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ABSTRACT

This document series has been established as part of Unesco's Science and Technology Programme to encourage an international exchange of ideas and information in science education. This volume addresses the improvement of biotechnology education in school up to year 10 and aims at improving the quantity and quality of biotechnology taught in schools worldwide. The five chapters deal with the following subjects: an overview of biotechnology; the principles and applications of biotechnology; the social implications of biotechnology; and considerations for implementation. In addition to discussing the history and basic principles of biotechnology, the book provides detailed classroom lessons that can be incorporated into many general science and biology programs. The lessons on the science of biotechnology are complemented by discussions of ethical and policy issues related to biotechnology, and these discussions are developed further in the chapter on the social implications of biotechnology and the educational context in which they should be considered. The book concludes with an overview of common barriers to the introduction of biotechnology into the curriculum and with suggestions for overcoming those barriers. (PR)

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Teaching Biotechnology in Schools

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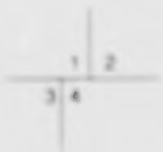

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Teaching Biotechnology in Schools

Edited by Joseph D. McInerney

Commission for Biological Education,
International Union of Biological Sciences (IUBS)

Section of Science
and Technology
Education

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Paris, 1990

Dedication

The authors, the editor and the Commission for Biological Education dedicate this work to our late colleague Dr. William V. Mayer, who died on 30 June 1989, shortly before the round-table conference he was to chair in Asendorf (Federal Republic of Germany). Bill organized the conference as a prelude to this publication, which he was to edit. For thirty years, Bill was an articulate and tireless advocate for quality science education around the world. The international science education community is diminished by his death, but bolstered by the enduring quality of his work and the lasting memory of his companionship.

Preface

The document series has been established as part of Unesco's Science and Technology Education Programme to encourage an international exchange of ideas and information in science education. This volume, developed by the Commission for Biological Education of the International Union of Biological Sciences (IUBS), addresses the improvement of biotechnology education in schools up to year 10 and aims at improving the quantity and quality of biotechnology taught in schools worldwide. The five chapters deal with an overview of biotechnology, the principles and applications of biotechnology, the social implications of biotechnology, suggestions for teaching biotechnology and considerations for implementation.

In addition to discussing the history and basic principles of biotechnology, the book provides detailed classroom lessons that can be incorporated in many general science and biology programmes. The lessons on the science of biotechnology are complemented by case studies for the classroom analysis, for upper grades, of ethical and policy issues related to biotechnology. Those case studies are supported by an entire chapter devoted to the social implications of biotechnology and the educational context in which they should be considered. The book concludes with an overview of common barriers to the introduction of biotechnology into the curriculum and with suggestions for overcoming those barriers.

This work is based largely on the results of a round-table conference on biotechnology education, held in Asendorf, Federal Republic of Germany, from 27 - 30 August 1989. The Conference was supported by IUBS and Unesco. In addition to the authors and editor, the following individuals attended the conference and contributed to the conceptualization of the final product: Raitcho Dimkov, Clement Ohrid University, Sofia, Bulgaria; C. H. Diong, Institute of Education, Singapore; B. Ganguly, National Council of Educational Research and Training (NCERT), New Delhi, India; L. Horst Grimme, University of Bremen, Federal Republic of Germany; Nils Oker-Blom, University of Helsinki, Finland; Wolfgang Sittig, HOECHST A.G., Frankfurt, Federal Republic of Germany; and Wu Xiang-yu, Peking University, Beijing, China.

Unesco wishes to thank Jospeh D. McInerney, Biological Sciences Curriculum Study, Colorado, United States of America, for his dedicated work in editing the work of the international contributors to produce an internally consistent document.

The opinions expressed in the following pages are those of the authors and not necessarily those of Unesco.

The plates which follow are colour versions of certain black and white illustrations in Chapter 4.

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Plates III, IV and V. Cellulose decompositions of cigarette paper in a damp chamber. Plate III shows the control after 6 weeks, Plate IV decomposition after 3 weeks and Plate V decomposition after six weeks. (Figure 40, page 75.)

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Plate IX. Comparing the streptomycin sensitivity of microbial strains, above *Bacillus subtilis*, below *Saccharomyces cerevisiae*. (Figure 22, page 143.)

Plate X. Inhibiting and encouraging growth of micro-organisms by the use of bactericidal substances. (Figure 23, page 145.)

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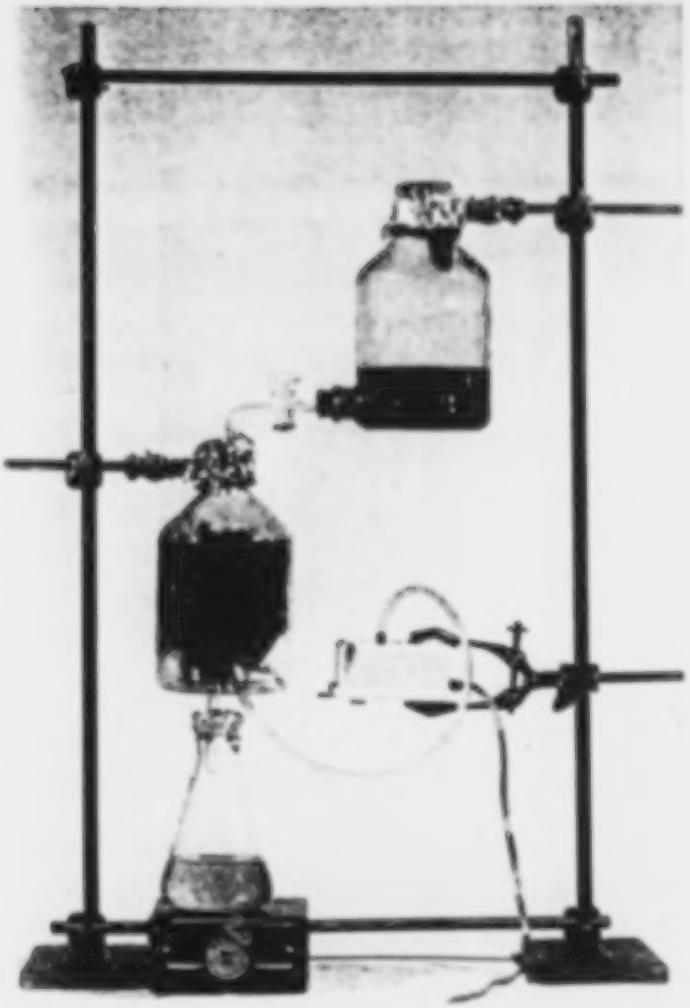

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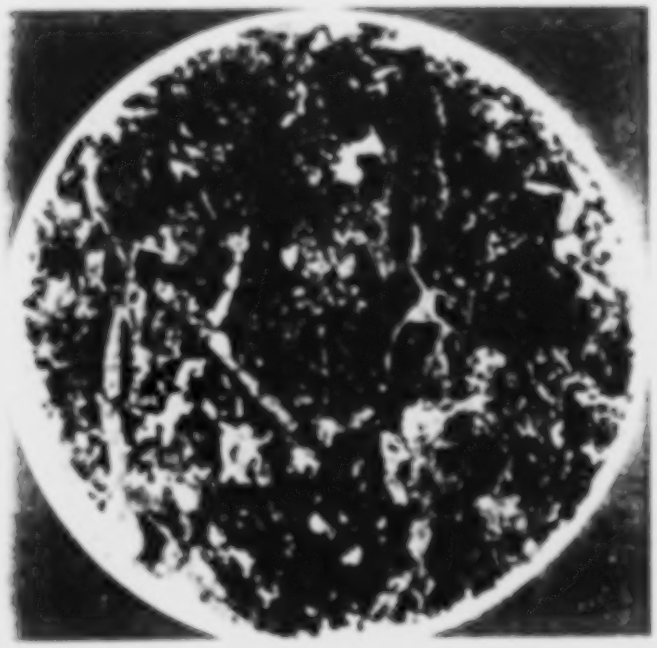

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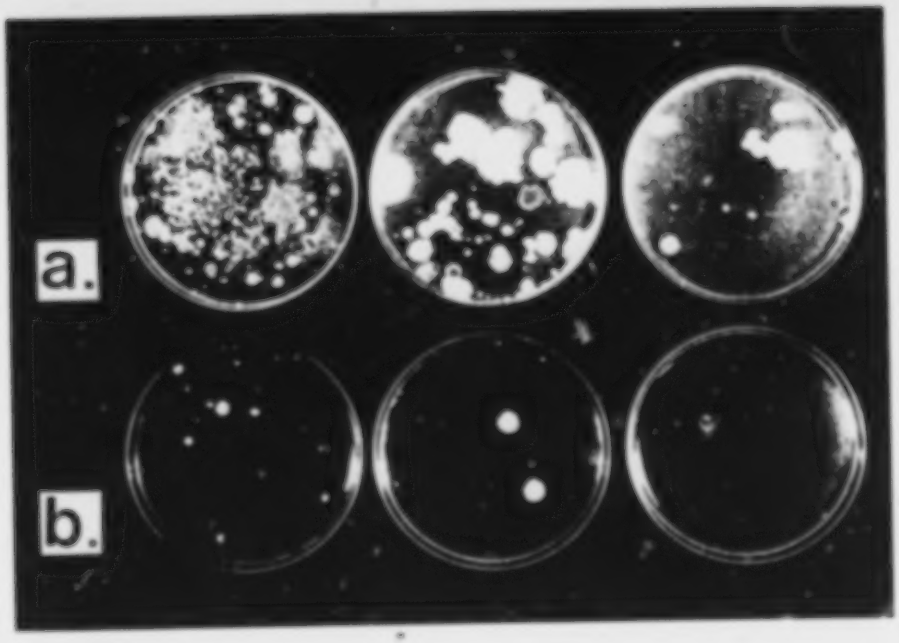

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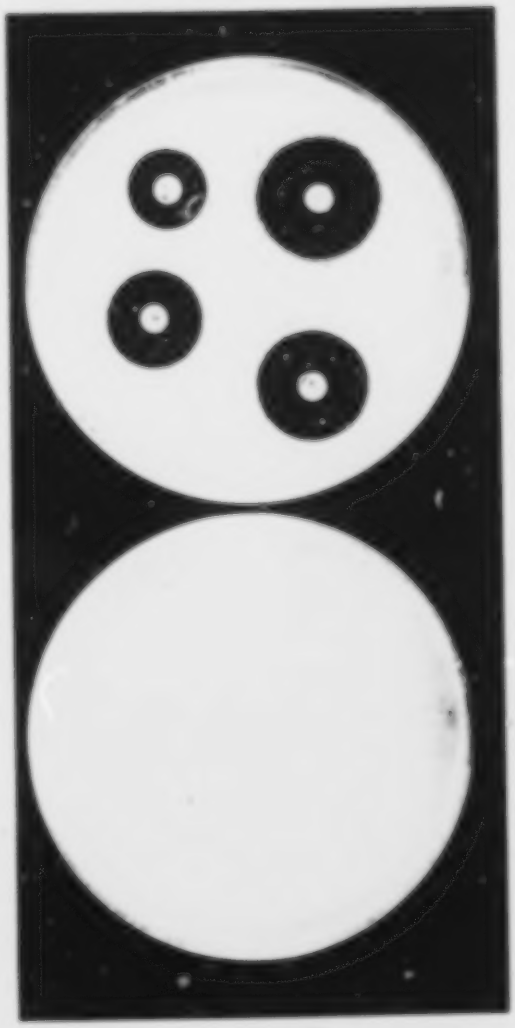

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Foreword

Only rarely in the history of science has an area of investigation moved so rapidly from basic research to application as has biotechnology, and particularly that aspect concerned with DNA technology. Since the discovery of restriction enzymes in the early 1970s, and their subsequent application in the manipulation of genetic material, the world has been witness to a stunning parade of progress in health care, industrial and agricultural applications, and research into enduring questions in basic biology.

This progress occurs against a backdrop of an emerging global economy, profound political changes that hold the prospect for improved international scientific cooperation, and a growing crisis in global environmental quality, all of which can be influenced substantially by continued progress in biotechnology. Unfortunately, the growth of biotechnology also coincides with a growing international consensus that general scientific literacy is inadequate. Indeed, basic concepts in biotechnology--and their personal and social significance--are addressed inadequately, if at all, in most precollege science curricula.

The purpose of the present publication is to help remedy that inadequacy. This publication, developed under the auspices of the Commission for Biological Education (CBE) of the International Union of Biological Sciences (IUBS), had its genesis in a series of papers prepared by CBE members and others between 1987 and 1989. In August 1989, CBE and IUBS sponsored a round-table conference in Asendorf, FR Germany, during which the participants used the existing papers and new information to outline a document that would provide background information and specific instructional strategies for teaching biotechnology in the secondary grades. The five authors then assumed responsibility for preparing the new chapters that comprise this book.

The preparation of this publication--by authors in several different countries and an editor in yet another--was itself an exercise in international cooperation, and a test for the universality of computer language and the dependability of fax technology. I am most grateful to the authors for their hard work, cooperation, and patience, to Dee Miller, at BSCS, for production of the final manuscript, and to Gerhard Schaefer, chair of CBE, for his constant support and encouragement. CBE and IUBS are confident that the material in this publication will help improve both the quantity and quality of instruction in biotechnology in secondary schools throughout the world.

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Colorado Springs, Colorado, USA
April 1990

Chapter 1

AN OVERVIEW OF BIOTECHNOLOGY

Introduction

Only three decades ago, one seldom heard the term biotechnology. Rarer still was the inclusion of biotechnology as a discipline of study at an educational institution. Since its emergence in the early 1970s, however, biotechnology has made tremendous strides, gaining ever-increasing significance as a field of basic research and through its important implications for health care and commerce. These important advances in biotechnology are due largely to recent advances in instrumentation and technology and to their subsequent application in extending the frontiers of biology as a science of life and as a basis for many industries.

Progress has been most spectacular in ecology, genetics, microbiology, molecular biology, biochemistry, cell-culture technologies, and process engineering. Many recent discoveries in these fields already have found useful applications in a wide array of industries such as food, fertilizers, fuels, chemicals and pharmaceuticals, crop disease, and pest control. Although still in its infancy, biotechnology has demonstrated its huge impact on humanity in both intellectual and practical terms; no other area of scientific research has advanced so rapidly in so short a time.

The most significant factor behind the rapid growth of biotechnology is the extraordinary progress made in the fields of molecular biology, genetics, and genetic technology. Studies of nuclei of individual cells led to the discovery of chromosomes and their behavior, to the identification of DNA as the genetic material, and subsequently to the discovery of the double-helical structure of DNA. Because of continuing breakthroughs in our understanding of DNA during the last few decades, we are now able to manipulate the genetic codes of various microorganisms.

The discovery of restriction enzymes in the early 1970s, and their application to genetic manipulation, resulted in the production of useful and valuable biologic products such as insecticides, vaccines, human insulin, human somatostatin, human growth hormone, interferons, and antibodies. Restriction fragment length polymorphism (RFLPs) can help to detect the presence of genetic disorders such as Huntington disease, cystic fibrosis, sickle cell anemia, muscular dystrophy, thalassemia, and hemophilia. It also is possible to use microorganisms as vectors to introduce new genetic messages into animals and plants to alter their characteristics and improve their breeds. For example, viral vectors can be used to introduce the gene for a missing enzyme into target cells as a possible way to correct metabolic disorders. Similarly, the use of recombinant interleukin-2 can help in the treatment of cancer, and advances in molecular biology have helped cancer specialists distinguish the mechanisms of oncogenes from those of tumor-suppressing genes--a second

major category of genes implicated in human cancers. Genetic manipulation also can improve crop yield, nutritional quality of seed crops, and the resistance of crop plants to pest, pathogens, and environmental changes and can increase milk and meat production by animals. These successes have encouraged the industrial use of bacteria, yeasts, filamentous fungi, plant and animal cells, and enzymes to produce commercially a wide variety of products.

Genetically engineered microbes now form the basis of many revolutionary processes that are helping to reinvigorate, diversify, and change the character of our traditional land-based, biologically based, and health-related industries, with subsequent impact on agricultural practices, medical research, waste management, and pollution control. The literature abounds with examples of industrial activities where biotechnologies have either replaced, or are in the process of replacing, traditional patterns of technology. Such cases include the manufacture of textile products, synthesis of flavorings and seasonings, production of ethanol, biogas, and hydrogen, and extraction of metallic elements. In fact, biotechnological methods already are used in the mass production of yeasts, algae, and bacteria to provide proteins, amino acids, vitamins, and scores of other useful products more economically. The use of biotechnology to develop new and improved crop varieties is advancing rapidly worldwide, a phenomenon that even developing counties with an agricultural base cannot afford to ignore.

In view of its universal applicability and usefulness in everyday life, biotechnology is now increasingly recognized as a potent tool in future development of both rural and urban economies in developed and developing countries. Many nations including Australia (CSIRO, 1981; Badger, et al., 1982); Great Britain (Spinks, et al., 1980; Beardsley, 1982; Anon, 1982a); Canada (Brossard, et al., 1980; Anon, 1983); France (Jaffe, 1982; Anon, 1982b); West Germany (Ingersoll, 1982; Anon, 1982c); Japan (Rogers, 1982); USA (Freeman, 1982); and O.E.C.D. (1983) are promoting biotechnology both politically and financially and have, in fact, prepared plans to encourage its use in industry and to improve the quality of life. Accordingly, there is much speculation about the industrial and market potential of various products of biotechnology. Some of these products are, in fact, already on the market (Schuuring, 1982; Anon, 1982d). A report from the Office of Technology Assessment (OTA, 1982) in the USA claims that more than 100 compounds--representing 17 different product categories ranging from amino acids to viral antigens--have a current market value in excess of 27 billion dollars. It is reasonable to assume that many more discoveries and products for industrial applications have emerged since publication of the OTA report. In fact, Sheets (1982) already has reported that a group of US chemical consultants has predicted market growth for biotechnology's products from US 25 million dollars in the early 1980s to US 65,000 million dollars by the year 2000. Glick (1982), on the other hand, estimated a rise of US 40,000 million dollars for a group of 500 products during the same period. Other sources (Batchelor, 1982), however, consider the returns from biotechnology to be much less certain and rather more distant. Nevertheless, growth in business firms, industries, research establishments, and other organizations has been

phenomenal, especially in the US. Indeed, biotechnology has attracted large investments and financial resources to develop and exploit new products and processes to serve various human purposes. That some of those ventures have failed is not, however, a reflection on biotechnology and its prospects, but rather the result of under-capitalization, inexperience, and poor business management. Those problems may be rectified by the acquisition of smaller genetic engineering firms by large, multinational corporations (Barinaga, 1990).

On the intellectual front, Leeney (1982), Gatz, et al. (1983), and Childs, et al. (1989), have reported significant and successful research activities in medicine and in animal and plant sciences. The ability to manipulate biological systems has brought increased awareness about the long-term needs to exploit renewable resources as industrial raw materials to reduce the use of industrial energy and to maintain and improve the quality of the environment. It is difficult to predict the future of any scientific discipline, and this is particularly the case in biotechnology, which relies on so many different disciplines for its progress. Recent research and new techniques in genetic technology will contribute to our knowledge in areas such as the regulation of the genetic mechanisms, development and differentiation of organisms, the organization of the human genome, the nature of the immune response, evolution, sex determination, and the relatedness of species.

Social Implications

As with most areas of science, rapid progress in biotechnology has created new problems as it proposed solutions for old ones. Many of these problems derive from the unknown benefits and risks that result from our ability to alter our environment, and indeed ourselves. The growth of biotechnology in recent years has been so fast and dramatic that general public understanding of biotechnology and its implications has failed to keep pace with its progress (OTA, 1987; Research and Forecasts, 1987). The capacity to manipulate the genetic apparatus in both the laboratory and the field has given us creative powers that pose new dilemmas for ethics, law, and public policy (see Chapter 3). The more we learn about the nature of life and its secrets, the more we reduce living organisms to malleable structures open to experimentation and manipulation.

The uses of biotechnology, and the extent to which those uses challenge long-standing values, will depend on how we perceive ourselves with respect to other organisms. Our ability to tinker with life already has generated considerable debate about the extent to which humans should intervene in the evolution of other organisms and in various ecosystems. These questions have legal and ethical dimensions that often bring us into direct confrontation with traditional value systems. This is particularly so in the application of genetic engineering to humans, where some fear of the use of genetic engineering to create morally and physically "perfect" humans (Annas, 1989; Friedman, 1990), and others

fear a loss of autonomy as various genetically based tests are made mandatory by employers and insurance companies (Holtzman, 1989; Nelkin and Tancredi, 1989). Mayer and McInerney (1984), for example, fear that "reduction of intellectual and behavioral variability may lead to a situation where people are too easily controlled and manipulated, and where there is little tolerance for individual diversity." In 1983, a group of religious leaders in the US urged that humans should not "assume the prerogative of the creator" (and that) no individual, group, or institution can legitimately claim the right or authority to make such decisions on behalf of the rest of the species" (Norman, 1983). Capron (1983), on the other hand, feels that the proposals for a total ban on genetic engineering would prevent the development of useful techniques as well as potentially harmful ones. Clearly, our knowledge of various biological processes is still far from perfect, and there are many unanswered questions about safety, ethics, and the long-range effects of gene manipulation on human health, disease, and the quality of life in general. Although the answers to the biological aspects of these questions will be universal, the legal, ethical, and policy decisions will vary from country to country, depending upon goals for national development and upon socio-cultural and moral traditions. Ethical and legal dilemmas related to genetic engineering in humans likely will increase with the growing application of rDNA in medicine (Holtzman, 1989; Nelkin and Tancredi, 1989), and with cooperative, worldwide efforts to sequence the human genome (OTA, 1988; National Research Council, 1988; Stewart, 1989).

The implications for sound education, therefore, are clear: each country must provide its students and teachers with sound content about the science of biotechnology and with the skills and information to analyze rationally the personal and social implications of biotechnology within the ethical, cultural, and legal traditions of that country. A well-educated citizenry in each of the world's countries can help the dreams of biotechnology become reality in the improvement of the quality of life on Earth.

The Evolution of Biotechnology

The recent and rapid use of biotechnology has inspired considerable excitement, speculation, and worldwide interest about the development of a host of new products and processes to satisfy divergent human needs. In basic practice, however, biotechnology is not a new subject; its origins are rooted in antiquity, and in substance it relates to some very ancient human activities that exploit basic biological processes. Food gathering, preparation, and preservation, for example, were essential components of the culture and economy of early communities around the world.

The Neolithic Era (9000 BC) marks the transition from hunting and food gathering to food production, which involves cultivation of edible plants such as wheat, barley, millet, and rye, and also includes malting technology. Starting perhaps from an unexpected

discovery of fermentation, humans have continuously improved their ability to exploit different raw materials through the use of living organisms or their biological processes. Thus, the brewing industry has been known from ancient civilizations since 3500 BC, and production of wine, bread, cheese, and yogurt has been a common heritage in all cultures. Although often ignorant of the natural principles involved, farmers have long practiced the art of improving their crops through careful selection of seeds from desirable plants. Similarly, they succeeded in developing better breeds of domestic animals long before the work of Gregor Mendel became widely known. By working with phenotypes, animal and plant breeders have been able to produce chickens that lay more eggs, cows that give more milk, horses that run faster, sheep that produce more wool, fish that grow larger rapidly, and plants that resist disease and produce more fruit and seeds.

Over the years there has been a gradual transition from the use of traditional recipes and empirical methods to the scientific understanding of the processes that underlie them. Once the genetic basis of heredity was elucidated, the benefits of cross breeding or hybridization became apparent, and production of species with desirable traits became commonplace. Following the identification of DNA as the genetic material by Avery, McCarty, and MacLeod, and the work of Watson and Crick on the structure of DNA, we have learned much about how the genetic code is duplicated and distributed to offspring cells. Recent research has given us the ability to isolate genes from cells, duplicate the genes in culture, and introduce them into other cells. These techniques, collectively referred to as genetic manipulation, are significant because they have the potential to overcome some of the natural restrictions that limit the range of biological variation under conventional breeding methods.

Biotechnology, therefore, is in fact the re-evaluation and refinement of old knowledge and traditional practices in the light of better understanding of various biological processes and the availability of new technologies. Fermentation techniques, for example, originated as homecrafts and were partly responsible for a change from cottage industries to town-based crafts. Traditional biotechnologies in this field involved natural processes. Later, the process was based on mixed cultures of wild yeasts, bacteria, and fungi, followed by batch inoculation with selected microorganisms, and control of enzymatic reactions. Only recently, through the use of genetic technology, fermentation has become more efficient and continuous, acquiring high industrial capabilities.

The development of biotechnology is, therefore, not a sudden phenomenon. On the contrary, it has a long history that has involved almost all civilizations. Table 1 lists major milestones in the evolution of biotechnology.

Table 1. Milestones in the history of biotechnology.

15000 BC	Simple forms of fermentation
9000 BC	Alcoholic fermentation, bread, cheese, yogurt
3500 BC	Lactic acid fermentation; leavened bread

2000 BC-600 AD	Cultivation of grape vine and crop improvement
1100	Alcohol discovered by distillation
1521	Aztecs harvest algae from lakes as food source
1650	Artificial growing of mushrooms in France
1670	Microbial mining of copper in Rio Tinto, Spain
1680	Invention of light microscope
	Leeuwenhoek describes microbes, yeasts
1750	Commercial leeching of copper from sulphidic ores
1818	Discovery of fermentation properties of yeasts (Hansen)
1855	First interpretation of microbial antibiosis (Babes)
	Discovery of bacteria, *Escherichia coli*
1865	First account of genes [Mendel]
1876	Germ theory; fermentations are caused by living microorganisms [Pasteur]
1881	Microbiological production of lactic acid
1890	Alcohol first used to fuel engines
1892	Alolation of protoplasts [Klefcker]
1897	Role of enzymes in alcoholic fermentation [Buchner]
1900	First successful plant tissue culture [Hanning]
1903	Microbial mining of sulphidic ores
1910	Use of microbes for sewage purification systems
1912	Microbial production of industrial acetone, butanol, glycerol by fermentation
1915	Isolation of the first filterable bacterial virus [Twort]
1916	Discovery of immobilization of enzymes
1921	Isolation of insulin [Banting, Best]
1925	Systematic screening for antagonistic organisms from natural sources
1927	Study of genetic mutation by X-rays
1929	Discovery of penicillin, antibiotic activity [Fleming]
1933	Identification of first enzyme - diastase [Payen, Persoz]
1940	First isolation and clinical testing of penicillin [Florey, Chain] Isolation of cortisone
	Culture of undifferentiated tissues
	Manufacture of penicillin
1941	One gene-one enzyme theory [Beadle, Tatum]
1944	Discovery of streptomycin [Schatz, Waksman]
	Discovery of DNA as hereditary material [Avery, MacLeod, McCarty]
1947	Discovery of thiobacillus fero-oxidans
1948	Discovery of chlortetracycline [Dugger]
1949	Manufacture of B12, antibiotics
1950	Production of many new antibiotics
	Basic components of DNA [Chargaff]
	3-dimensional structure of alpha helix [Pauling, Corey]
	Discovery of jumping genes [McClintock]
1952	The Hershey - Chase experiment
1953	Discovery of double helix structure of DNA molecule [Watson, Crick]
1954	Cultivation of isolated cells [Muir, Hildebrandt, Riker]
1956	Discovery of DNA polymerase
	Discovery of transfer RNA
	Discovery of biological synthesis of RNA and DNA
1957	Discovery of interferon [Isaacs, Lindenmann]
	Production of SCP from hydrocarbons
1960	Discovery of messenger RNA

1961	DNA probe [Marmur, Doty, Schildtkaut]
1963	Discovery of causative agent [acidophilic bacterial] in mineral leaching
1968	First isolation of gene by hybridization techniques [Kohne]
1970	Discovery of restriction enzymes [Arber, Smith, Nathans]
	Discovery of reverse transcriptase [Temin, Mizutani, Baltimore]
1972	DNA recombination techniques [Jackson, Seymours, Berg]
	Research on embryo transfers
	First applications of immobilized enzymes
	First somatic hybrid plant
1973	First successful genetic engineering experiments [Cohen, Boyer]
1974	Localization of T1 plasmid [Schell, Van Montagu]
1975	Asimolar conference on possible dangers of genetic engineering
	Discovery of hybridoma and monoclonal antibodies [Milstein, Kohler]
	Sequencing of genes [Coulson, Sanger]
1976	Discovery of endorphins [Guillemin]
	Development of high fructose corn syrup
1977	Cloning of first human protein - somatostatin
1980	Cloning of human interferon [Weissmann]
	Genetically engineered insulin used for diabetic treatment
1982	Commercialization of first genetically produced veterinary vaccine
1983	First product of genetic engineering - Humilin - goes on sale
	Synthesis of gene for growth hormone, production of human insulin, interferon, cattle growth hormone.
	First use of an RFLP as a marker for a hereditary disorder - Huntington disease [Gusella, et al.]
1984	Databank on hybridomonas, human growth hormone, human serum albumin, pig dysentery vaccine, pesticide resistant strains.
1985	Vaccines, ethanol, aliphatic compounds
1986	Salt osmotic regulation, improvement of photosynthesis, amino acids enzymes,
1988	Other improvements in nitrogen fixation; isolation of the gene for Duchenne muscular dystrophy
1989	First insertion of a foreign gene into humans; isolation of the gene for cystic fibrosis; sequencing of the human genome begins

According to Smith (1986), there are four major phases in the development of modern biotechnological practices:

1. *Food and Beverage Biotechnology.*

   This includes processes related to brewing, wine making, and leavening of bread that have been practiced for several thousand years; the Sumarians and Babylonians were drinking beer by 6000 BC, and the Egyptians were making leavened bread by 4000 BC. That these processes were being carried out by microorganisms--namely the yeast *Saccharomyces*--was not recognized until the seventeenth century, with the development of the light microscope. Similar biotechnologies using microbiologically based processes include production of fermented milk products, soya sauce, and various other oriental foods.

2. *Products Initially Developed from Microbiological Processes under Non-sterile Conditions.*

By the end of nineteenth century, a number of substances including ethanol, acetic acids, butanol, and acetone were produced on a commercial scale. Production employed simple fermentation under selective microbial control through careful manipulation of the environment; complex, mechanical systems were involved. Use of these processes for the production of said substances declined with the development of the petrochemical industry. Recent escalation in oil prices, however, has led to a resurgence in the deployment of fermentation practices. These processes also are associated with waste-water treatment, decomposing, and detoxification of human sewage.

3. *Sterile Practices in Biotechnological Processes.*

With the introduction of new engineering practices in the 1940s, the pathway of biotechnology changed; allowing the mass cultivation of a microorganisms in pure form in fermenters. Thus, by the prior sterilization of the medium and the fermenters, and through technological provisions to exclude contaminants, it has been possible to use only selected biocatalysts--microbes, enzymes, and plant or animal cells, for example--for industrial production of antibodies, enzymes, aminoacids, polysaccharides, vaccines, and other products. Unfortunately, many such technologies are complicated and expensive, and hence are suitable only for high-value products.

4. *New Dimensions for Biotechnology.*

Modern biotechnology has resulted from:

a. fundamental discoveries in the life science, particularly the discovery of the role of DNA as the carrier of the genetic code;
b. gene-manipulation techniques to create new life forms;
c. microbial techniques for cultivation, screening, and selection of useful cells or microorganisms, and manipulation of their behavior under controlled conditions;
d. immobilization of enzymes and whole cells for biocatolytic purposes;
e. cell- and tissue-culture techniques to accelerate propagations of useful plants; and
f. down-stream-processing techniques for extraction, treatment, purification, and conversion of useful materials following biomass production.

The most important aspect of the modern biotechnology will be its capacity to use agricultural by-products and other organic wastes to produce more valuable

products. Biotechnology therefore holds reasonable promise to reduce the scientific and technological gap between the developing and industrialized countries.

Definitions and Working Concepts of Biotechnology

The term "biotechnology" is said to be a contraction of "biological technology." Primarily, biotechnology is concerned with the application of biological knowledge, and hence it has a very wide connotation. As Vohra (1981) points out,

> Biotechnology could refer to various processes involving the use of bacteria for the manufacture of alcohol, yogurt, and insulin...or methodologies to improve production and preservation of food or techniques used in extracting concentrates from plants and animals for drugs and medicines, or even the manufacture of fibers from biological materials for making cloth.

In practice, though, biotechnology utilizes bacteria, yeast, fungi, algae, plant cells, and animal cells as essential components of industrial processes.

Recent research in the field has opened new dimensions for the advancement and application of biotechnology in industry, and hence it is now variously defined, with several countries having their own official versions. The Industrial Biotechnology Association (1984) regards biotechnology simply as the use of living organisms to make commercial products. According to Ganguly (1989), biotechnology is the selective use of biological information to influence both reproduction and industrial production. Mayer (1986) characterizes biotechnology as an industrial use of living organisms or their components, such as enzymes. Rao (1986) used the term as a broad base, synonymous to biological technology, and defined it as "a method or procedure wherein the biological process of a microbe, plant or animal cells, and tissues are used to obtain either the whole organisms or their constituents or their metabolic products." Smith (1986) refers to biotechnology as "the science of applied biological processes, involving the practical application of biological organisms, systems, or processes to manufacturing and service industries and to environmental management." Gregory (1986) looks upon biotechnology "as the use of cells for making useful products or doing valuable things with or without genetic engineering being involved."

The Commission on Biotechnology of the International Union of Pure and Applied Chemistry (IUPAC, 1981) describes biotechnology as "the application of biochemistry, biology, microbiology, and chemical engineering to industrial processes and products

(including here the products in health care, energy, and agriculture) and on the environment." The European Federation of Biotechnology (1981) sees it as "the integrated use of biochemistry, microbiology, and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissues, cells, and parts thereof." The British consider it as "the applications of biological organisms, systems, or processes to manufacturing and service industries (Markley and Robins, 1985). In *Biotechnology: A Development Plan for Canada* (Brossard, et al., 1980), biotechnology is described as "the utilization of a biological process, be it microbial, plant or animal cells, or their constituents to provide goods and services." In the Dutch context (Biotechnology: a Dutch Perspective; 1981, as quoted by Bull, et al., 1982a), it is described as "the science of applied biological process. The science of the production processes based on the action of microorganisms and their active components, and of production processes involving the use of cells and tissues from higher organisms." According to this, medical technology, agriculture, and traditional crop breeding are not generally considered to be biotechnology. In Australia (Badger, et al., 1982), biotechnology is "the devising, optimizing, and sealing-up of biochemical and cellular processes for the industrial production of useful compounds and related applications." This definition regards biotechnology as embracing all aspects of processes of which the central and most characteristic features are the involvement of biological catalysts. Plant agronomy falls outside this definition.

The foregoing definitions tend to emphasize traditional processes underlying brewing and baking. Recombinant DNA technology, which is largely responsible for revolutionizing biotechnology in modern society, is not covered explicitly by most of the definitions. The Japanese, on the other hand, see biotechnology more in terms of its social utility and market potential. Thus they hold it as "technology using biological phenomena for copying and manufacturing various kinds of useful substances." Similarly, the National Science Foundation (NSF) in the USA defines biotechnology as "the controlled use of biological agents, such as microorganisms or cellular components, for beneficial use." These definitions suggest that technology is always beneficial. It is true that technology has provided us many solutions, but in doing so it has also created many problems.

The term "biotechnology," of course, combines "bio" and "technology"; the former, implies life processes and relates to biology as the science of life, while the latter refers to a variety of tools, techniques, principles, and processes as part of technology. In our growing modern culture, science and technology represent two basic human activities, that while separate, are interrelated in our daily life. Science is an effort associated with natural human desire to know, to understand, to explain, or predict some natural phenomenon. Technology, on the other hand, is the result of an equally natural human desire or endeavor to find ever new and better ways to satisfying human needs and to achieve human purposes in the socioeconomic, cultural, and ethical context using knowledge, materials, resources, and energy.

Foecki (1974) has diagrammatically summarized the contrasts between these two areas, showing for each the "motive force" that drives the activity, the "process" that characterizes it, and the "product" thereof. Foecki's diagram is reproduced as figure 1.

With respect to the motive forces, one is essentially a desire to know, the other a desire to do; they are, indeed, different. The confusion, however, arises in their relationship, because to do something better, it is necessary to know something more. This relationship simply implies that the product of one activity becomes an input for the other, although the activities remain distinct.

In relation to the process, the term research in science characterizes the activity of seeking knowledge and understanding and hence refers to the application of scientific methods to understand some particular phenomenon. In simple language, research may imply organized attempts to explain and to find cause-and-effect relationships among phenomena. In this sense, the meaning of research or scientific methods does not cover the processes of designing or problem solving, which characterize technology.

Science moves from the particular to the general, trying to discover ever broader relationships to explain individual phenomenon, while technology proceeds in the opposite direction, using general principles to solve specific problems. Science asks, "What is?" (For example, is there a relationship between smoking and lung cancer? What is the effect of vitamins on human health?). Technology asks, "What should be?" (For example, given my age, health, and economic means, should I smoke, and if so, what and how much? Considering cost, availability and other factors, which fertilizer should be more effective on my crops?)

Turning now to the products of science and technology, the result in the former case is an incremental increase in the total reservoir of human understanding--the organized body of knowledge that enables us to understand, explain, and predict both natural and human-made phenomena. Technology involves two types of products. In most cases, the optimum solution to a problem involves nothing essentially new; the solution is merely some variation of a standard or prototype solution. Sometimes, however, innovative solutions or techniques arise, and once they prove useful, they become part of the reservoir upon which future designers can draw in solving similar problems. Thus, technology may be akin to a reservoir of techniques, processes, devices, and materials that have demonstrated their usefulness in accomplishing particular tasks. One can view technology, therefore, as a problem-solving process that seeks to satisfy human needs by using available resources and knowledge. The process acknowledges constraints that arise from the limitations of resources, knowledge, or natural laws, as well as impediments imposed by sociocultural and economic factors.

Thus analyzed, biotechnology may simply mean the use of living things to make products to satisfy human needs or achieve human purposes. As with any technology, the

Understanding - Explaining - Finding Out

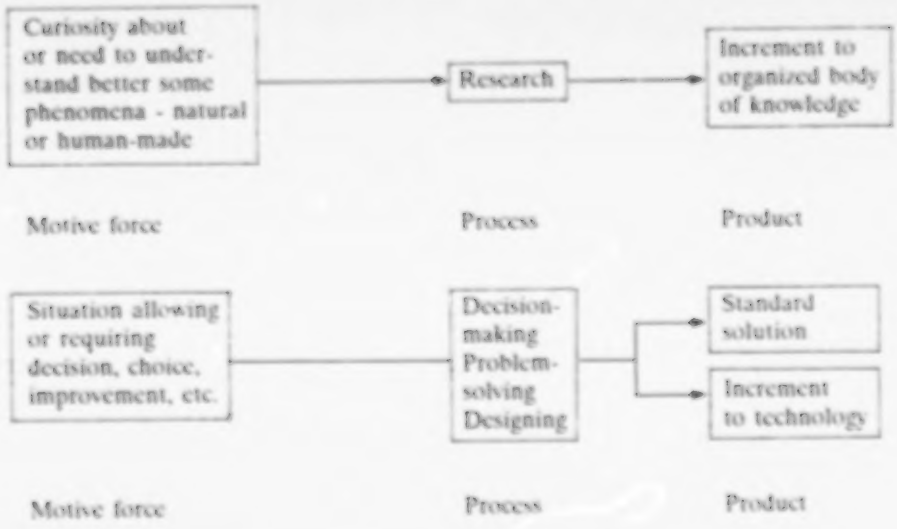

Figure 1. Contrast between science and technology as a part of natural human activities.

pursuit of biotechnology produces some unintended or unanticipated negative consequences.

In the light of emerging new knowledge and its potential applications, the Office of Technology Assessment (OTA, 1982) divides biotechnology into "old" and "new." The old implies a broad perspective favored by the Europeans, that is, the use of living organisms to produce products considered useful to humans; new relates to industrial uses of DNA, cell fusion, and bioprocessing. In commenting on this definition, Kiefer (1987) emphasizes two points. First, biotechnology does not refer to one procedure, one technique, or one process, but rather encompasses diverse methods for using living organisms or their products to prepare items for the marketplace. Second, biotechnology entails the combination of two disciplines of science and engineering. Furthermore, the new OTA definition confines itself to the commercial side of DNA science only.

To provide a common basis for comparison among the O.E.C.D countries, Bull, et al. (1982b) have defined biotechnology as "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services." The "scientific and engineering principles" in the definition are said to cover a wide range of disciplines, although there is a heavy reliance on microbiology (including microbial technology), genetics and gene technology, and biochemistry. Similarly, the term "biological agents" includes a wide range of biological catalysts such as enzymes, microorganisms, animal, and plant cells. The term "materials" also is all-embracing, covering both inorganic and organic substances. Furthermore, "goods" implies all the products of industries concerned with food, beverages, pharmaceuticals, and biochemicals, while "services" relates largely to water purification, industrial and domestic waste management, and the production of energy. This definition tends to reconcile various concepts implied in other definitions and is hence more comprehensive and universally acceptable.

As an educational tool, one can view biotechnology as an interdisciplinary or a multidisciplinary subject that includes an interwoven network of activities that reflect the complex interaction of its various component and disciplines, and the areas of application in industry. Biotechnology, therefore, employs techniques derived from chemistry, microbiology, genetics, biochemistry, medical sciences, chemical engineering, and computer science. The underlying component in its development is the ability of scientists and engineers to understand each other's language. Like any technology, biotechnology operates within the socioeconomic and cultural environments that may stimulate or constrain its progress and public acceptability. To be successful, its practitioners must develop innovative and optimal operation of processes where biochemical catalysis plays a fundamental and irreplaceable role.

The definition by Bull, et al. makes clear that the ultimate success of biotechnology depends upon advances in fundamental scientific disciplines. The authors further believe

that short cuts, empiricism, and superficial attention to basic scientific and technological principles and processes are likely to lead to poor process performance at best, and to expensive failures at worst.

Educational Implications of Biotechnology

Because of the ever-increasing significance of biotechnology, universities around the world already offer a variety of courses on the subject, and the number of students enrolling in those courses is increasing. Although much has been written about the need to introduce biotechnology into school curricula, relatively little has been accomplished.

There have been some successes, particularly in the United States of America, the United Kingdom, and some other industrially advanced countries, where efforts are in progress to introduce biotechnology concepts in a variety of courses in the school curriculum and to provide special in-service training programs to prepare teachers for teaching these concepts. Elsewhere, however, biotechnology has not been introduced as a subject of study in schools. It is possible that teachers lack both awareness and the training necessary for teaching the subject. Furthermore, fear of the unpredictable potential of biotechnology, as well as the lack of confidence and acceptability on the part of general public, have impeded the inclusion of biotechnology in school education. An additional obstacle may be that schools have no easy access to literature explaining what biotechnology is, which of its concepts can easily be taught through existing school disciplines, and how some practical activities can be designed to teach its basic techniques safely in school laboratories in most countries.

Biotechnology is akin to other high-tech subjects that involve high cost and hence are not easily approved for school teaching. Moreover, biotechnology is a complicated, multifaceted subject; many school teachers find it rather difficult to handle in the classroom. Biotechnology combines science and technology, and often raises socioeconomic, political, and ethical issues. Most teachers, however, are competent at traditional instructional methods and in teaching science as a body of knowledge. They lack confidence in handling problem-solving approaches and in structuring discussions of controversial issues, two strategies that are crucial for effective education about biotechnology. (Chapter 5 addresses barriers to the introduction of biotechnology in schools.)

Applications of biotechnology are now wide spread. As part of our daily life, it affects the food we eat, the waste we generate, the energy we consume, and the way we live. It is vital for sustaining and improving national economies. Given proper education and training in the contributing disciplines of genetics, microbiology, biochemistry, and molecular biology, and development of relevant research programs in plant-genetic

manipulation, any nation can advance its own needs and may be able to promote international developments. To achieve this, schools must play a major role in educating future citizens about the value and potential of biotechnology.

Recent reports and reviews on education emphasize that science and technology education need significant reform. School curricula in general are designed to prepare students for entry into universities rather than for a life of work; such training often ignores national development objectives. Many school graduates lack the knowledge and skills to assume their civic responsibilities and realize their potential in the growing culture of science and technology.

School-based biology ranges from nature study involving elementary observations of common animals and plants, to the study of form and function of organisms, and the basic principles of genetics, ecology, and cellular and molecular biology. Instruction often focuses on the *acquisition* of knowledge rather than on how knowledge is *discovered* and *applied* in meeting various human needs. Because the industrial applications of biotechnology are excluded from the school biology curriculum, problems related to improvement of the quality of life and available technological solutions for personal development and community improvement are often ignored in the teaching of school biology. For the same reason, biologically related social, cultural, political, ethical, and economic issues are conveniently omitted from classroom discussion. Such omissions are unfortunate, because the school biology curriculum must grow out of human events and aspirations if it is to be relevant and useful for the learner. It is essential, therefore, that the school biology curriculum be revised in the light of recent advances in biotechnology. Such revisions should occur in a context that promotes the teaching of biology:

- in socioeconomic and interdisciplinary context;
- as an evolved and evolving body of knowledge;
- as a process of enquiry and problem solving; and
- as a partner of technology in the service of humanity.

There is, of course, a limit to what the curriculum can accommodate, and we cannot simply add new topics--whatever their significance--to the already over-crowded biology curriculum. Biotechnology, however, can easily be inserted into the existing segments of microbiology, molecular biology, genetics, biochemistry, and chemistry; this is a matter of reorienting and updating those topics. With some care and ingenuity, the underlying principles and processes of biotechnology can be taught in the context of the traditionally held indigenous technologies and associated cultural values.

Biotechnology in schools should not, however, be confined to biology, chemistry, or technology. Its multidisciplinary and cross-curricular dimensions can make important contributions to a variety of courses in the humanities, social studies, and home economics, which provide appropriate forums for the discussion of the controversial socioeconomic

and ethical issues raised by biotechnology.

If today's students are deprived of a basic understanding of biotechnology, they will be ill-suited to assume their civic responsibilities and make knowledgeable decisions in controlling and directing progress of biotechnology as a servant of humanity rather than its master. Biotechnology must, therefore, become an essential component of both formal and non-formal education at all levels.

Themes and Concepts for Biotechnology Education in the Schools

The study of school-based science curricula reveals that they already contain the fundamentals of all the important areas of biotechnology. This possibly led the Royal Society Working Group (1981) to remark that biotechnology, both theoretical and experimental, can be introduced into schools without removing any essential elements of current syllabuses or further overloading them. This can be accomplished through the judicious use of material that exemplifies basic principles, develops skills, and generates awareness of application in industry.

1. *Themes or Concepts Appropriate for Biotechnology Education.*

A. Microbiology

1. The variety and diversity microorganisms with reference to habitat, size, form, structure, physiology, reproduction, multiplication rates, and life-histories of organisms.
2. Not all microorganism are not harmful or pathogenic; many are very useful.
3. Some microorganisms can be grown in large quantities to produce useful products from a wide variety of raw materials; the process sometimes generates large quantities of waste materials.
4. Microorganisms need suitable raw materials for growth.
5. The use of microorganisms in industry, with special reference to: production of beer, wine, spirits, vinegar, proteins, vitamins, and antibiotics; recycling of nitrogen and other nutrients; sewage and water treatment, as well as waste management; production of gas and liquid fuels as an alternative to finite fossil fuels; applications in the dairy industry.
6. It is possible to produce a cheaper, better, or different product by selecting more suitable alternative organisms, changing the raw materials, or improving the engineering process.

7. General microbiological techniques, with an emphasis on safety.
8. Study of a cow's stomach as a fermentation tank that converts green plant materials to milk; bacteriocidal and bacteriostatic methods.

B. Molecular biology

1. Cell structure and function
2. Cell nucleus and its function
3. The structure of DNA and RNA
4. DNA replication
5. The genetic code
6. Protein synthesis
7. Recombinant DNA
8. Isolation of cell organelles and their products

C. Genetics

1. Basic principles of genetics
2. Chromosomes as the physical bases of heredity
3. Improvement of crops and domestic animals through selective breeding
4. Hybridization
5. Linkage and recombination, leading to the understanding of spatial relationship of genes on the chromosome
6. Crossing over

D. Biochemistry

Enzyme structure and function
Recognition, isolation, and purification of biological molecules

E. Engineering

Basic principles in relation to developing cost-effective biological processes, with special reference to aseptic operations, reactor design (fermenters and immobilized biocatalysts reactors), product recovery and marketing, and the use of simple instrumentation and process control.

F. Theory and practical skills in biotechnology

1. Recombinant DNA technology
2. Monoclonal antibody technology
3. Bacterial plasmids

4. Restriction enzymes
5. Use of DNA probes
6. Electrophoresis
7. Fermentation
8. Physical processes for separation and purification of biological products
9. Safety measures and biological containment
10. Cleaning and sterilization

G. Cell biology

1. Animal and plant cells
2. Cell theory
3. Cell reproduction and growth
4. Cell or tissue culture (it is possible to grow complete organisms from a single cell; cell and tissue culture techniques may be used for storing germ plasm of useful genetic varieties)
5. Cell cloning

H. Applications of biotechnology

1. Human applications (vaccines, diagnostics, gene therapy)
2. Agricultural applications (natural pesticides, growth hormones)
3. Industrial applications (energy, waste management, methane production)
4. Ecological applications (natural biological control)

I. Basic concepts in genetic technology (excerpted from BSCS, 1988)

1. Genetic rearrangement occurs naturally.
2. Natural genetic rearrangement is one source of the variation that occurs in nature.
3. Microorganisms can act as vectors (carriers) for genetic information.
4. Genetic rearrangement results in the expression of new traits.
5. Genetic material is universal in the living world.
6. Experiments that involve potentially biohazardous material must be conducted in accordance with established safety measures.
7. Molecular biologists have learned how to control some of the processes of natural genetic engineering.
8. New techniques allow biologists to cut apart DNA and put it together in new combinations (recombinant DNA).
9. Recombinant DNA gives organisms new characteristics.

10. Recombinant DNA technology allows biologists to produce valuable biological products.
11. Not all the information in DNA is translated into mature mRNA.
12. Many genes exist in pieces, with coding regions (exons) separated by non-coding regions (introns).
13. The ability to manipulate genetic material quickly and efficiently raises important ethical, legal, and policy questions.
14. All organisms contain nucleic acid.
15. The genetic code is a universal language.
16. We can measure the evolutionary relatedness of two organisms by comparing their DNA; greater variation indicates more distant relationship.
17. Genetic technology is a powerful research tool that has improved our understanding of basic biological principles.
18. Progress in genetic engineering raises important and complex ethical issues for individuals and society.
19. Ethics is the study of right and wrong behavior and of the rules that guide behavior.
20. Reasonable people may disagree about what constitutes right and wrong behavior.
21. Ethical analysis is a process of critical thinking; it is based on sound facts.
22. Often times there are no clearly right or wrong answers to ethical dilemmas.
23. Ethical positions often have implications for public policy. Public policies must generally meet several tests before they are likely to be successful.
24. Human DNA is highly variable; no two people have the same sequence of bases.
25. Variation in base sequences can create or eliminate recognition sites for restriction enzymes.
26. If we digest (chop up) the DNA from two people with the same restriction enzyme, the variation in the DNA will result in different-sized fragments for each person.
27. These fragments--called RFLPs--can be used to detect the presence of disease-causing genes.
28. The genes for some disorders such as sickle cell anemia can be detected directly. This requires knowledge of the specific mutation and restriction enzyme that cuts DNA at the mutation site.
29. Pieces of DNA that are on the same chromosomes are said to be linked.
30. During meiosis, homologous chromosomes exchange DNA in a process called crossing over.

31. During crossing over, pieces of DNA that are closely linked will be separated less frequently than pieces of DNA that are distantly linked.
32. Some RFLPs are closely linked to disease-causing genes.
33. If one can detect such an RFLP, one can predict that the disease-causing gene is also present.
34. The predictive power of linked RFLPs depends on how closely linked they are to the disease-causing gene.
35. Linked markers allow detection of disease-causing genes in the absence of knowledge about the underlying cause of the disease.
36. The ability to detect disease-causing genes prenatally and in heterozygotes raises important ethical and legal questions.
37. Recombinant DNA technology permit the manipulation of genetic material in the laboratory.
38. Plasmids can be used as vectors to carry new genetic information into a bacterium.
39. Laboratory tests can determine whether the desired trait has been transferred to the bacterium.
40. Experiments that involve recombinant DNA must be conducted in accordance with established safety procedures.

J. Some general concepts

1. Biotechnology has been used in the service of humanity since early human history.
2. Changes in the genetic message lead to variation in natural populations.
3. This variation can be accelerated and controlled by human intervention.
4. One aspect of modern biotechnology involves splicing genes into a bacterium to produce a desired protein to combat disease or to develop a new product.
5. Gene therapy involves insertion of normally functioning genes to replace defective genes.
6. Biotechnology can create as well as solve problems, and hence it should be guided by knowledgeable persons and decisions.
7. Biotechnology is in a state of flux, and students should be prepared for changes in both goals and techniques as more knowledge is acquired. Recent research has generated the possibility of many new products and services, and has provided the technology to produce old products in new ways. Further research will enhance these possibilities. The extent to which these possibilities become realities depends on complementary developments in process engineering and on favorable commercial and socioeconomic conditions.

8. The relative economic importance of various aspects of biotechnology varies from country to country, and there are wide differences between economically developed and less developed countries.
9. The use of living organisms in an economic context can sometimes give rise to social, ethical, political, and legal issues that must be examined alongside the scientific and economic aspects of a potential development of biotechnology.
10. School-based education in biotechnology can be tied to the production of goods and services, in particular to local industries related to food technologies, dairy products, greenhouse technologies, pest control, sewage treatment, or water treatment.
11. The development of biotechnology produces career opportunities that require a wide range of knowledge, aptitudes, skills, qualifications, and experience.

The Association for Science Education, in the U.K. (1986), has studied the ways to incorporate biotechnology into a contemporary science syllabus. The results are shown in tables 2, 3, and 4.

Table 2. Incorporation of biotechnology into the biology syllabus (Association for Science Education, 1988).

SYLLABUS TOPICS	REFERENCE TO BIOTECHNOLOGY
BIOLOGICAL RESOURCES and their exploitation.	Ethanol (gasohol), methane (biogas); biomass
CELL BIOLOGY SCP;	Fermentation processes; nutrient requirements; cell culture.
COORDINATION. Nervous control.	Use of plant hormones. Insulin production-genetic and hormonal engineering. Sources of hormones used in medicine.
CROP AND LIVESTOCK PRODUCTION/BREEDING	Agriculture - embryo transplants; genetic improvement.
DIVERSITY OF ORGANISMS	Social, economic and technological impact of micro-organisms.
ECOLOGY	Microbial pesticides. Batch and continuous fermenters.

ENERGY -including biomass and nutrient cycles; energy flow through food chains.	Ethanol; methane; biomass.
ENZYMES	Enzymes as products of fine organic chemicals with many industrial uses; enzyme inhibitors.
EVOLUTION - artificial selection.	Selection of variants in antibiotic production; the emergence of resistent strains; drug resistance.
FOOD PRODUCTION - processing.	Dairy, fish and meat products; beverages; bakers including yeast; food additives; novel foods; mushrooms; amino acids; vitamins; starch products; glucose and high fructose syrups; yogurt manufacture.
HEALTH and HYGIENE - including immunity and vaccinations.	Antibiotics; diagnostic agents (enzymes, antibodies), enzyme inhibitors; steroids; vaccines.
HEREDITY/GENETICS/DNA, genes, chromosomes, engineering.	Recent advances in molecular biology in relation to biological industrial processes, e.g. the use of genetically engineered microbes to make insulin and human growth hormone.
MINERAL NUTRITION	Cell and tissue culture techniques; mineral, e.g. water cultures requirements of microbes for suitable growth in fermenters.
PEST CONTROL	Microbial pesticides.
PHOTOSYNTHESIS, e.g. efficiency of energy conversion.	Biomass.
REPRODUCTION	Clones. Culture techniques.
RESPIRATION	Ethanol production - as an industrial chemical in the alcoholic drinks industry.
SAPROPHYTES	Sewage disposal; pollution control; ensilage and, e.g. decomposition; recycling; composting processes. Carbon and nitrogen cycles.
SYMBIOSIS/MUTUALISM	Rhizobium and other nitrogen fixing bacterial inoculants; yogurt manufacture.
VARIATION	Artificial selection techniques, including genetic engineering in relation to a number of products.

Table 3. Incorporation of biotechnology into the chemistry syllabus (Association for Science Education, 1988).

SYLLABUS TOPICS	REFERENCE TO BIOTECHNOLOGY
CHEMISTRY OF LIVING THINGS	Genetics/recombinant DNA
ECONOMICS OF CHEMICAL PROCESSES	Use of enzymes of conventional catalysts/chemical reactions (lower temperatures; fewer strong reagents; perhaps cheaper and safer).
ENERGY - fuels and resources;	Biomass; gasohol; biogas catalysis. Use of enzymes.
FOOD AND AGRICULTURE	a. Dairy industry - cheese; yogurt b. Novel foods c. Improved crops - disease resistance, higher yields d. Pest control - use of fungi; genetic engineering e. Preserving foods - pickling
METALS - extraction from ores	Use of microorganisms to leach out metals.
NITROGEN/AMMONIA	Nitrogen-fixing bacteria/reduction in quantities of fertilizers and energy used c.f. chemical fixation of nitrogen.
SOCIAL IMPLICATIONS	a. Novel foods, e.g. mucoprotein, SCP b. Pollution and its control c. Recycling of materials d. Health and hygiene - antibiotics; immunology/monoclonal antibodies; synthetic hormones; new vaccines; interferons
WATER	Water purification. Reservoir to water works to mains supply. Used water treatment. Sewage works to river.

Table 4. Incorporation of biotechnology into the science syllabus (Association for Science Education, 1988).

SYLLABUS TOPICS	REFERENCE TO BIOTECHNOLOGY
DISEASES AND THEIR TREATMENTS	Antibiotics; immunology/monoclonal antibodies; synthetic hormones; new vaccines; interferons.
ECOSYSTEMS	Importance of bacteria, fungi and algae, the nitrogen cycle; nitrogen-fixing bacteria.
FUELS AND ENERGY	Biomass, gasohol and biogas, use of enzymes as catalysts. Fermentation and alcohol production photosynthesis - ways of increasing the efficiency of plant to absorb and utilize sunlight energy and minerals.
REPRODUCTION, GROWTH AND CELLS	Genetic engineering. Cell and tissue culture.
SCIENCE AND SOCIETY	Genetics. Plant and animal breeding. Economics of processes. Comparison of the use of conventional plant with one using microorganisms.
THE EARTH AS A SOURCE OF MATERIALS	Biomass; formation of oil/gas by bacteria; extraction of metals from ores by microorganisms; renewable sources of materials when supplies of oil, gas and coal run out.
VARIETY OF LIFE	Microorganisms; the importance of enzymes.

Bayrhuber (1989) divides themes in biotechnology into two groups. One, shown in figure 2, is based on the use of the natural processes of living organisms or cell components. The other, shown in figure 3, relates to the use of living organisms modified by gene technology.

Formal biotechnology should not be introduced in the very early grades. If, however, the school is situated in an area where there are suitable local industries, it might be useful to teach biology as an industrial base in the context of environmental studies.

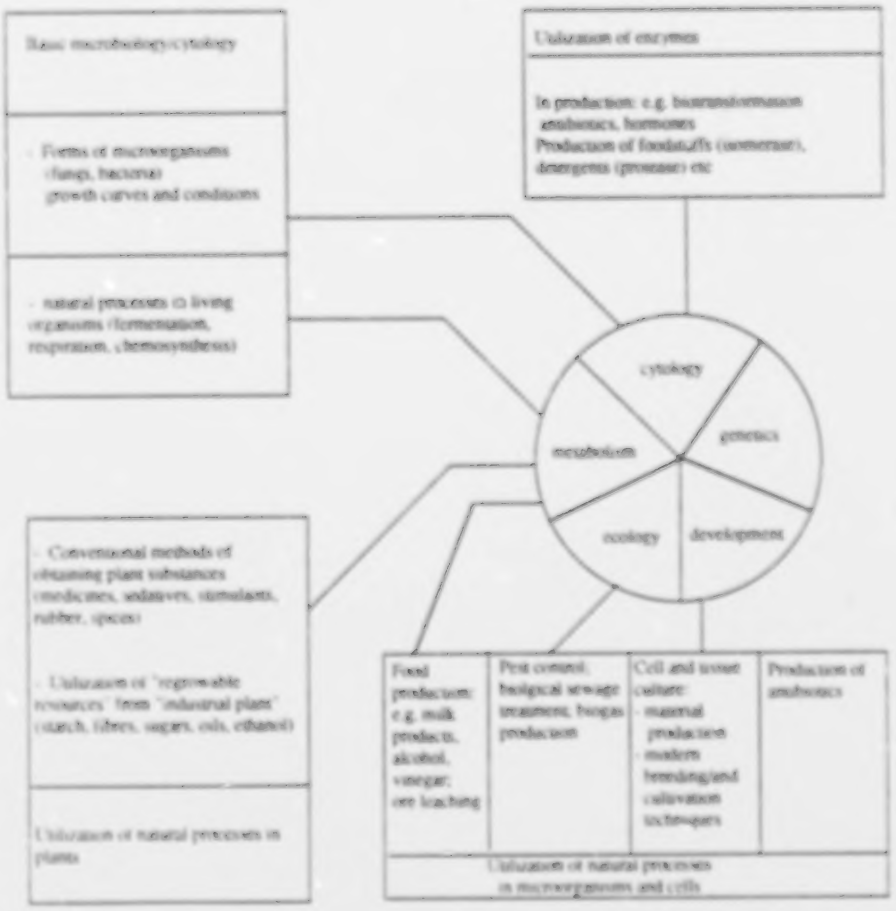
Basic microbiology/cytology
- Forms of microorganisms (fungi, bacteria) growth curves and conditions
- natural processes in living organisms (fermentation, respiration, chemosynthesis)
Utilization of enzymes
In production: e.g. biotransformation
antibiotics, hormones
Production of foodstuffs (isomerase),
detergents (protease) etc
cytology
genetics
microbiology
ecology
development
- Conventional methods of obtaining plant substances (medicines, sedatives, stimulants, rubber, species)
- Utilization of "regrowable resources" from "industrial plant" (starch, fibres, sugars, oils, ethanol)
Utilization of natural processes in plants
Food production: e.g. milk products, alcohol, vinegar, ore leaching
Pest control, biological sewage treatment, biogas production
Cell and tissue culture:
- material production
- modern breeding and cultivation techniques
Production of antibiotics
Utilization of natural processes in microorganisms and cells

Figure 2. Possible biotechnology themes dealing with the use of natural processes in living organisms or organelles, and the general biology topics in which they can be covered (in higher grades of secondary education).

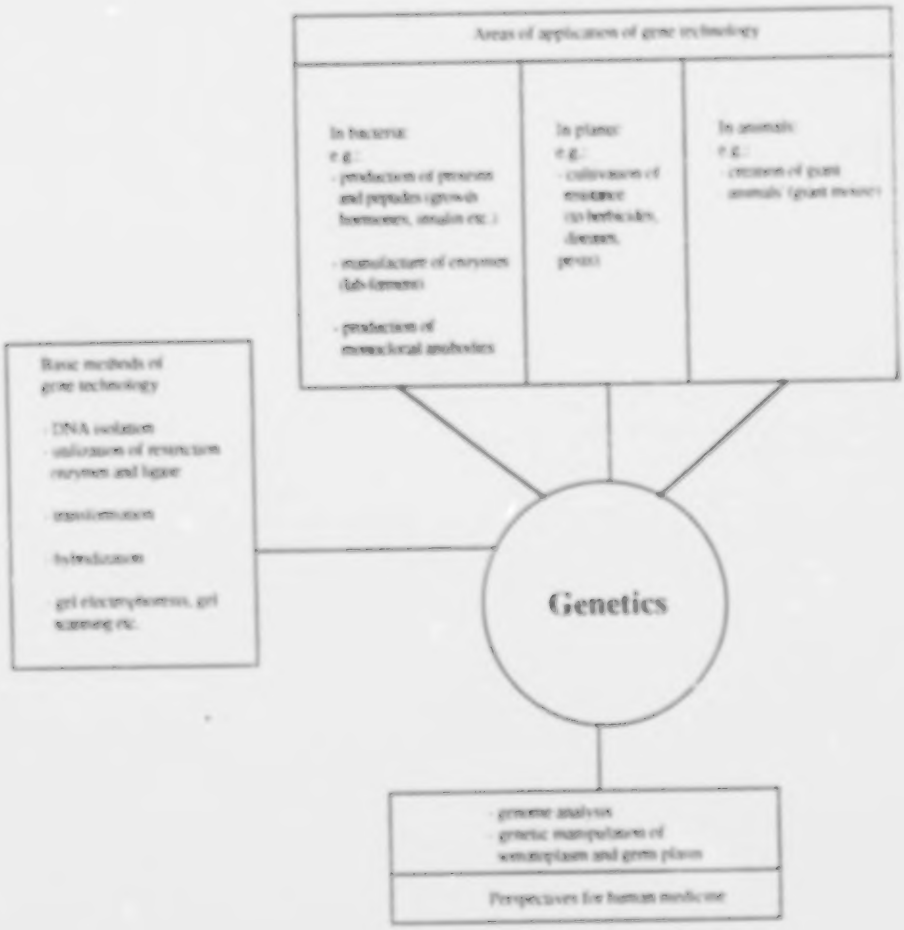
Areas of application of gene technology
In bacteria:
e.g.:
- production of proteins and peptides (growth hormones, insulin etc.)
- manufacture of enzymes (lab-ferments)
- production of monoclonal antibodies
In plants:
e.g.:
- cultivation of resistance (to herbicides, diseases, pests)
In animals:
e.g.:
- creation of giant animals (giant mouse)
Basic methods of gene technology
- DNA isolation
- utilization of restriction enzymes and ligase
- transformation
- hybridization
- gel electrophoresis, gel scanning etc.
Genetics
- genome analysis
- genetic manipulation of somatoplasm and germ plasm
Perspectives for human medicine

Figure 3. Possible biotechnology themes for genetics in the higher grades of secondary education dealing with the use of organisms modified by gene technology.

In middle grades, students can easily study simple biotechnology involving the use of living organisms in the industrial production of food and alcoholic beverages, and in waste management. At higher grades, where science is offered as separate disciplines, basic biotechnological principles and techniques may be taught in the context of those disciplinary structures. One should not, however, overload the already over-crowded science syllabus.

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Chapter 2

PRINCIPLES AND APPLICATIONS OF BIOTECHNOLOGY

Cell Structure and Physiology

The most striking developments in biotechnology have come from the manipulation of the metabolism and genetic features of cells. This has led to ever deeper studies into the structure and physiology of cells of all types--microbial, plant, and animal.

Structurally, cells fall into two groups, procaryotic and eucaryotic, the principle difference being whether or nor the hereditary material, deoxyribose nucleic (DNA), is compartmentalized in a cell organelle called a nucleus.

1. *Procaryotic Cells.*

The cells of bacteria and cyanobacteria (blue-green bacteria) are procaryotic. They are small (1-10 mm), usually with a protective cell coat secreted by the cell membrane proper. There is no nucleus. The single, circular chromosome lies free in the cell cytoplasm and there is some extra-chromosomal DNA contained in a self-replicating plasmid vesicle. There are few other organelles and no cytoskeleton. Although the structure of procaryotic cells is simple, as shown in figure 1, they exhibit a very wide range of biochemical processes, both aerobic and anaerobic.

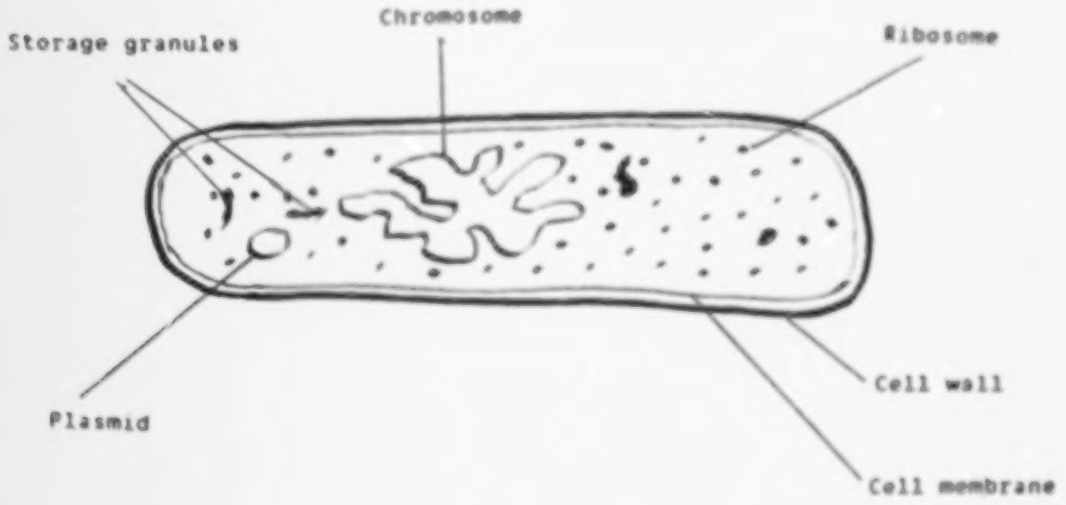

Figure 1. The structure of a procaryotic cell.

2. *Eucaryotic Cells.*

The cells of fungi, plants, and animals confine the hereditary DNA in a nucleus. The hereditary DNA is arranged in many long, linear strands within the nucleus and is wrapped into compact chromosomes by proteins called histones. Associated with several of the chromosomes is a distinctive structure called a nucleolus, in which ribosomal ribose nucleic acid is packaged and transported through pores in the nuclear membrane into the cytoplasm, where it performs an important role in the synthesis of proteins.

In contrast to the cytoplasm of procaryotes, the cytoplasm of eucaryotes is highly structured and contains several types of organelles, as shown in figure 2.

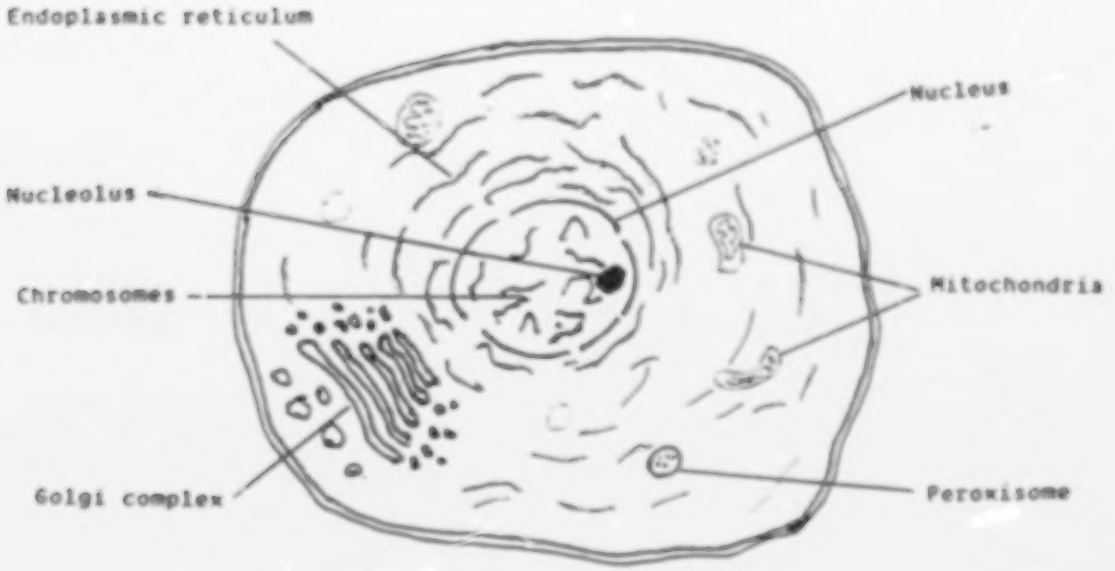

Figure 2. The structure of a eucaryotic cell, including organelles.

A cytoskeleton of actin filaments, intermediate filaments, and micro-tubules is attached to the organelles and the plasma membrane of the cell. These filaments keep the organelles in their relative positions in the cell and bring about their movements during various phases of the cell cycle. They also can effect changes in cell shape that may lead to cell locomotion.

A. Organelles

The cytoplasm of eucaryotic cells contains a number of membrane-bound organelles. Prominent in the cytoplasm of differentiated cells is the endoplasmic reticulum (E.R.), seen in sections as a network of membranes forming the boundary of flattened vesicles called cisternae. The endoplasmic reticulum is probably a single, heavily folded membrane, and most likely is continuous with the nuclear membrane. Suspended in the cytoplasm are a large number of ribosomal subunits. At any one time, some of these subunits become attached to the outer surface of the E.R. as polyribosomal complexes, giving the reticulum a rough appearance in electron micrographs. Some parts of the reticulum form tubules rather than cisternae, and in these regions ribosomes are not attached, giving the endoplasmic reticulum a smooth appearance, which contrasts with the rough endoplasmic reticulum.

The nuclear membrane is a double membrane containing numerous pores that provide channels for the exchange of proteins and ribosomal subunits with the cytoplasm. At the same time, the nuclear membrane keeps the nucleoplasm free of cytoplasmic filaments, organelles, and some of the larger molecules.

Golgi apparatus. Another cytoplasmic organelle is the Golgi apparatus, especially prominent in secretory cells. The Golgi complex is made up of a series of flattened cisternae bounded by a single membrane (figure 3). The number of the cisternae varies from one type of cell to another, and perhaps with the activity of the cell, but it is usually between six and ten. Associated with the cisternae are a large number of small vesicles. These vesicles are particularly clustered around the rim of the flattened cisternae, and between the Golgi complex and the cisternae of the endoplasmic reticulum. They are involved in the transport of proteins and lipids between the E.R. and the Golgi complex, and between the Golgi complex and the cell surface.

The endoplasmic reticulum, and its associated ribosomes, is the site of the biosynthesis of proteins and lipids for the reticulum itself and for other organelles, including the plasma membrane. A ribosome that is manufacturing a special signal peptide becomes bound to a receptor on the E.R. This results in a polypeptide chain being synthesized and translocated across the E.R. membrane. The soluble proteins destined for the lumen of the E.R. or other organelles pass completely into

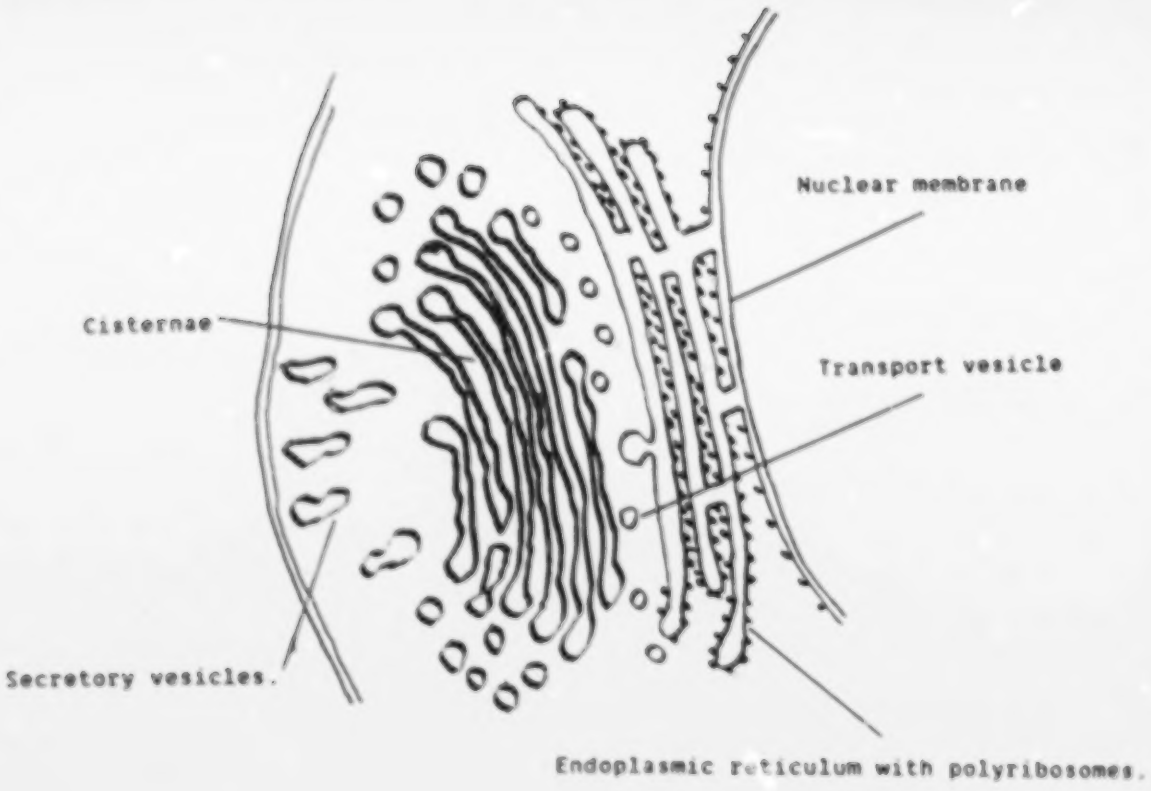

Figure 3. The Golgi complex and endoplasmic reticulum.

the E.R. lumen. Proteins destined for locations within the E.R. membrane itself or other cell membranes are not released into the E.R. lumen, but remain within the membrane. As soon as a ribosome stops making a signalled polypeptide, it is released from the E.R. membrane into the cytoplasm, and its place taken by another ribosome. Ribosomes freely dispersed in the cytoplasm make all other proteins encoded by the genome.

Proteins and lipids processed in the E.R. are carried to the Golgi complex in transport vesicles. The cisternae of the Golgi complex function as a sequential series of processing compartments. Transport vesicles, with their load of proteins and lipids, enter the complex on its inner face, where what is called the *cis* compartment is situated. They then pass from there through the medial cisternae to the *trans* compartment, formed by the distal cisternae. During their passage through these three compartments, the proteins undergo modifications that principally consists of a form of glycosylation. From the *trans* compartment, the proteins are moved to the trans Golgi network of the tubules, from where they are distributed to the plasma membrane, lysosomes, and secretory vesicles.

Mitochondria and chloroplasts. Mitochondria, and, in plant cells, chloroplasts, are concerned with providing ATP for the energy requirements of the cell. Both are bounded by a double membrane and both have their own DNA, as shown in figure 4. Current thinking holds that both were derived from parasitic microorganisms that entered eucaryotic cells early in their evolution. Their growth within the cell results from a combination of protein produced by the mitochondrial genome, and proteins provided by the nuclear genome and absorbed from the cytoplasm.

The mitochondrial outer and inner membrane enclose two separate compartments, the inter-membrane space and the internal matrix. The two membranes have different functions. The inner one is the site of ATP synthesis. The outer membrane is more permeable than the inner one, and therefore serves as a sieve to admit all molecules of 10,000 daltons or less, including small proteins. The inner membrane is impermeable to ions and selectively permeable to the small molecules admitted to the inter-membrane space. The composition of the two spaces, therefore, is chemically different.

The matrix space contains all the enzymes and the small molecules required in the respiratory chain for the generation of ATP. The inner membrane is heavily folded to form cristae. This gives a large surface area for the production of ATP in a very small volume, a nice illustration of the biological principle of form and function.

The chloroplast differs from the mitochondrion in two respects. In structure, the inner membrane of the chloroplast is not folded, but a third membrane, the thylakoid membrane within the stroma (equivalent to the mitochondrion matrix), is heavily folded. In electron micrograph sections of chloroplasts, this folding has the appearance of stacks

of flattened vesicles (the grana) spaced along the thylakoid membrane (figure 4). It is probable, however, that these are all interconnected and form part of a single, much-folded membrane. This membrane and the space it encloses contains the photosynthetic, light-absorbing system, the enzymes for the electron transport chain and ATP synthesis.

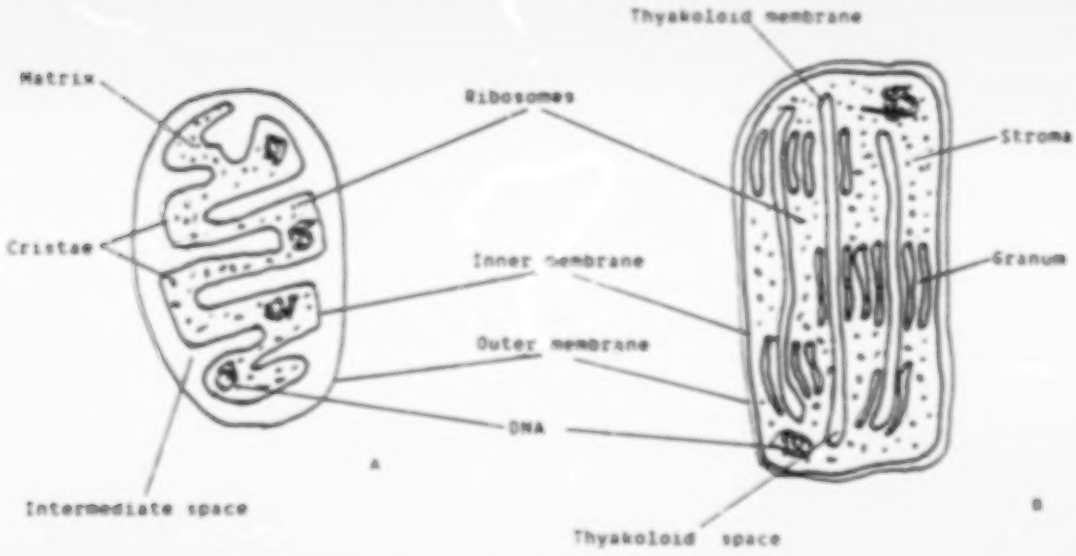

Figure 4. A. Mitochondrion. B. Chloroplast.

This leads into a second difference between chloroplasts and mitochondria, which is one of metabolism. The energy to generate ATP and ultimately the synthesis of carbohydrate from CO_2 is derived from light energy, whereas in mitochondria it is the oxidative phosphorylation of fatty acids and pyruvate that provides the energy for the synthesis of ATP.

Vesicles. Eucaryotic cells usually contain numerous vesicles. Some are temporary cell organelles that ingest materials from the environment, or discharge secretory products. Two vesicles enclosed in single membranes are more persistent. Peroxisomes (0.15-0.25u) are found in most cells. The proteins found in peroxisomes are derived from the cytoplasm, as they have no DNA of their own. The composition of the contents of peroxisomes is extremely variable, differing from one cell type to another, and in the differing states of cell activity. The main constituents of these vesicles are enzymes, and their function is to carry out oxidative reactions to detoxify various toxic materials in the cell. Peroxisomes are therefore prominent in liver and kidney cells, but they are widely distributed in animal and plant cells. They use both molecular oxygen and hydrogen peroxide for detoxification; excess peroxide is broken down by the catalases they contain.

The other small, persistent vesicles found in most cells are the lysosomes. These contain a wide range of hydrolytic enzymes with an optimum pH of 5. These enzymes digest microorganisms and macromolecules that may enter the cell, and they are probably involved in the controlled digestion of aged cytoplasmic organelles.

3. *Enzymes.*

The numerous biochemical reactions that take place in a living cell could not take place in the rapid, coordinated, and specific way necessary to maintain the cell in equilibrium without the intervention of enzymes. Enzymes are proteins that have a catalytic function. The three dimensional folding of the basic amino-acid chain gives each enzyme a unique, irregular surface that provides a high degree of specificity for specific substrate molecules. The greater part of the roughly spherical enzyme molecule is not directly involved in its catalytic activity, but serves to hold the active sites in their critical three-dimensional form on the surface of the molecule.

These active sites combine with the substrate. Small changes in the shape or stereochemistry of the substrate molecules interfere with the enzyme action. Some enzymes are highly specific, catalyzing the breakdown of one particular substrate molecule. Others are less specific and will break down a family of molecules, but perhaps not all of them with the same efficiency. In such cases there will be competition for active sites. For example, the conversion of succinic acid into fumaric acid is catalyzed by the enzyme succinic dehydrogenase. If malonic acid is added to the reaction mixture, it will inhibit the conversion because the structure of malonic acid is sufficiently similar to succinic acid to compete with it for active sites. In this instance, the inhibition is reversible by the addition of excess succinic acid.

The ratio of enzyme to substrate concentration is an important factor in determining the velocity of enzyme-catalyzed reactions. An increase in the substrate concentration from a low level in the presence of a given concentration of enzyme will increase the rate of substrate breakdown at first, but as more substrate is added the increase falls off until a steady rate is reached. This relationship is shown in figure 5. This effect again depends on the availability of active sites. Measurement of the velocity of enzyme action in cell preparations gives useful information about the behavior of enzymes. Many biochemical reactions are linked to one another so that the reaction products of one enzyme are the substrate for another enzyme. In such chain reactions (as in respiration) the enzymes are localized in the cytoplasmic cytoskeleton, or within an organelle (mitochondria), so that the enzyme system benefits from substrate concentration.

Other factors that affect the efficiency of enzyme action are co-enzymes, prosthetic groups, and metal ions. Co-enzymes are small molecules that are loosely bound to the enzyme and assist its catalytic action through a particular chemical group it carries. Nicotinamide-adenine dinucleotide ($NADT^{+}$) is a co-enzyme that functions as a hydrogen

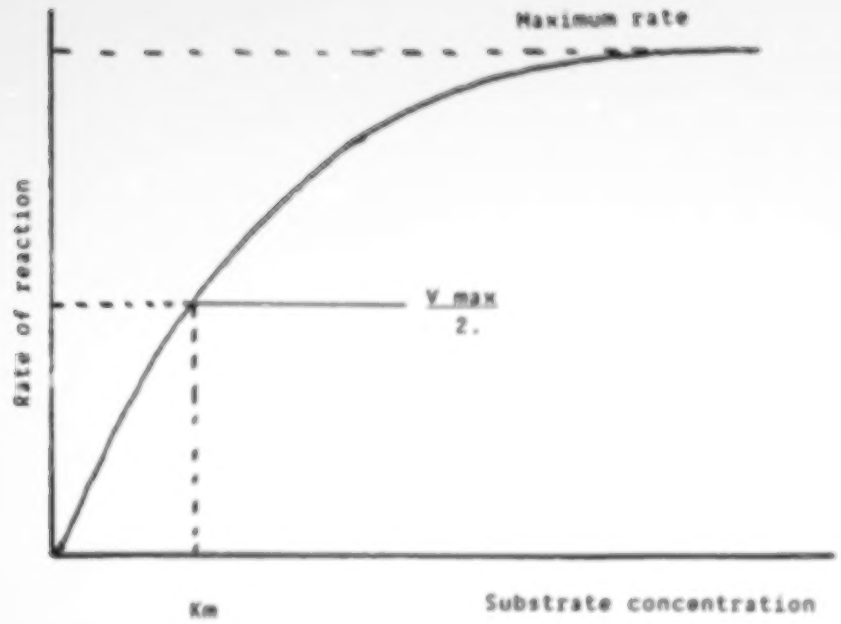

Figure 5. The relationship between substrate concentration and enzyme activity.

carrier in oxidation-reduction reactions. Prosthetic groups, on the other hand, are firmly bound to the enzyme. An example is flavin-adenine dinucleotide (FAD), which forms a flavoprotein enzyme that also catalyses certain oxidation-reductions. Many enzymes require metal ions as co-factors, hence the nutritional requirement of such metal ions as Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Fe^{++}, and Co^{++}.

Most enzymes have acidic and basic ions in the side chains, so that changes in the pH of the enzyme environment may affect these groups, and through changes in cross-linking alter the shape of the molecule and therefore its efficiency. Whatever the changes at the ultrastructural level may be, it is well-known that enzymes work most efficiently in a narrow pH range. Enzymes are denatured at extreme levels of pH.

4. *Nucleic Acids.*

By the beginning of this century, biologists had recognized that the material that transmits the hereditary information from one generation to another is within the chromosomes of the nucleus. At first it was thought that only protein molecules have enough complexity to carry the hereditary information, but by 1950 there was enough experimental evidence to show that deoxyribose nucleic acid (DNA) forms the information-containing units, the genes, in the chromosomes.

DNA is a long, unbranched polymer of four nucleotide bases: adenine, cytosine, guanine, and thymine. Each base is coupled to the sugar deoxyribose, and these units are linked in a chain by covalent phosphodiester bonds that join the fifth carbon deoxyribose group to the third carbon of the next. Two such strands are linked through the bases by hydrogen bonds to form a double helical polymer, as shown in figure 6. Guanine is always linked to cytosine, and adenine to thymine, as these pairs form the greatest number of effective hydrogen bonds.

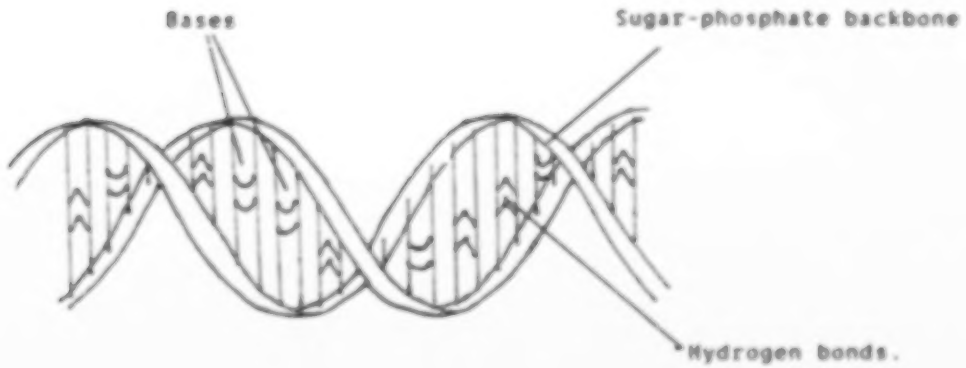

Figure 6. The DNA double helix.

It is now well established that DNA carries the hereditary information, but several different proteins are closely bound to the DNA and play an important part in its function. The protein linked to the DNA takes up stain in cytological light-microscope preparations and gives the combination of the two polymers the name chromatin.

These proteins fold the long DNA molecules, which may be 1.75 to 2.5 cms, protecting them from breakage and allowing their accommodation within the nucleus as chromosomes. A second function of the proteins is to regulate the activity of the genes by altering the form of the folding of the DNA strands. In eucaryotes, a unique class of proteins--the histones--folds the DNA into compact structures called nucleosomes. Non-histone proteins further fold the nucleosomes, packing them into looped domains (figure 7).

Figure 7. A. Nucleosomes. B. Chromosome loops.

A. Replication

Each time a cell divides, it is important that the entire collection of genes carried by its chromosomes is transmitted to the daughter cell. To bring this about the helical strands of DNA are separated by an enzyme that runs along one of the strands. As they are separated, two new complementary strands are created by the assembly of bases on the two original strands by the enzyme DNA polymerase. DNA polymerase can only be added to new bases from the 5' end of a strand to the 3' end. One of the strands of the opened helix, the so-called leading strand, starts with an exposed 5' end, so replication on the leading strand proceeds smoothly and rapidly. As figure 8 demonstrates, the other complementary strand, the lagging strand, starts with a 3' end. In this strand, the DNA polymerase lays down short blocks of complementary nucleotides in the 5'-->3' direction; these short blocks are subsequently connected by another enzyme system to complete the complementary strand. The lagging strand is replicated more slowly, and in an intermittent manner.

The genetic information in the DNA molecule is coded in the order of the nucleotide bases. It is therefore important that the copying during DNA replication is accurate. The accuracy is about one error in every 10^9 base-pair replications. "Proof-reading" mechanisms remove errors, thereby maintaining the fidelity of the code in replication at this level. Occasionally, however, an error occurs, and the linear order of the bases is altered. This new sequence is copied in subsequent divisions and thus perpetuated. Such errors alter the genetic code and thus constitute a mutation. The mutation will be lost if it results in the death of the cell. If it affects a non-essential gene it will be likely retained without effect. In rare cases it may be an advantage to the individual carrying the mutation and thus be perpetuated. Mutations contribute to the genetic variation upon which natural selection operates.

B. Transcription

The sequence of the nucleotide bases in the DNA molecule, which forms the genetic code, is not used directly in programming the synthesis of proteins in the cytoplasm. Instead, a copy of short lengths of the sequence (the genes) is made by a specific ribose nucleic acid (RNA) molecule--messenger RNA (mRNA). RNA, like DNA, is composed of a linear sequence of the same nucleotides, with the exception that uracil replaces thymine, and the sugar-phosphate backbone consists of ribose instead of deoxyribose sugar.

The RNA copy is made in much the same way as in DNA replication. Matching base pairs are formed on a region of the DNA template and covalently bonded with the aid of RNA polymerase to form an RNA chain, which contains all the coded information of the DNA. This process is known as transcription.

The RNA copy separates from the DNA template and passes out into the cytoplasm as a single strand, where it directs the synthesis of a specific protein coded in the gene that has been transcribed. The use of mRNA to carry the genetic information to the site of protein synthesis--instead of using the DNA directly--enhances the rate at which proteins can be produced. Thousands of RNA copies of a gene can be made and each copy used repeatedly in a very short time to synthesis the protein. The transcription of genes is controlled by gene-regulatory proteins, which allow some genes to be copied at a particular phase of the cell's metabolism, while others are not transcribed until required. Other RNA transcripts assist in the assembly in the cytoplasm of amino acids in a defined order to form proteins. These are transfer RNA (tRNA) and ribosomal RNA (r-RNA), whose function will be described later.

Early work on transcription was conducted on bacteria--procaryotes. Later, in studies on eucaryotes, research revealed that parts of the gene are non-coding.

These regions, called introns, separate the coding regions, called exons. The mRNA transcribed from eucaryotic DNA copies both the introns and exons; but before passage into the cytoplasm, the introns are excised from the mRNA, leaving the exons to code for the protein.

C. Translation

DNA and protein molecules are complex, folded, linear series of nucleotides and amino acids, respectively. Research in the 1960s showed that there is a code correspondence between these two linear assays. Triplets of nucleotide bases code for an amino acid (figure 9.) When translated, the sequential order of the nucleotides in the mRNA strand produces an ordered strand of amino acids. Because there are four nucleotides in the mRNA molecule, there are $4^3=64$ possible triplets (codons); but only twenty amino acids are found in proteins. Research soon demonstrated that most amino acids are coded by several codons; that is, the genetic code is degenerate.

The genetic code on the mRNA is translated by a multi-component ribosome that passes rapidly along the mRNA strand in the 5'-->3' direction only. Small transfer RNA molecules match amino acids to the appropriate triplet of nucleotides. One end of the molecule carries an anti-codon that pairs with a particular RNA triplet (or codon), and the other end carries a triplet that covalently links with a specific amino acid. A "start" signal of nucleotides on the mRNA strand ensures that the reading frame of the nucleotide bases is correct (figure 10).

As the polyribosome moves along the mRNA codon by codon, a tRNA molecule--with the matching anti-codon for the next codon on the mRNA--attaches itself to the polyribosome. The ribosome catalyzes the peptide bonding of each successive amino-acid residue activated by the tRNA. In this way, as figure 10 illustrates, the nucleotide sequence is translated into an amino-acid sequence one codon at a time. When a "stop" signal is reached, the polypeptide chain and the ribosome are released into the cytoplasm. The polypeptide chain becomes folded into a mature protein, and the ribosome assists in the synthesis of another polypeptide chain on an mRNA molecule.

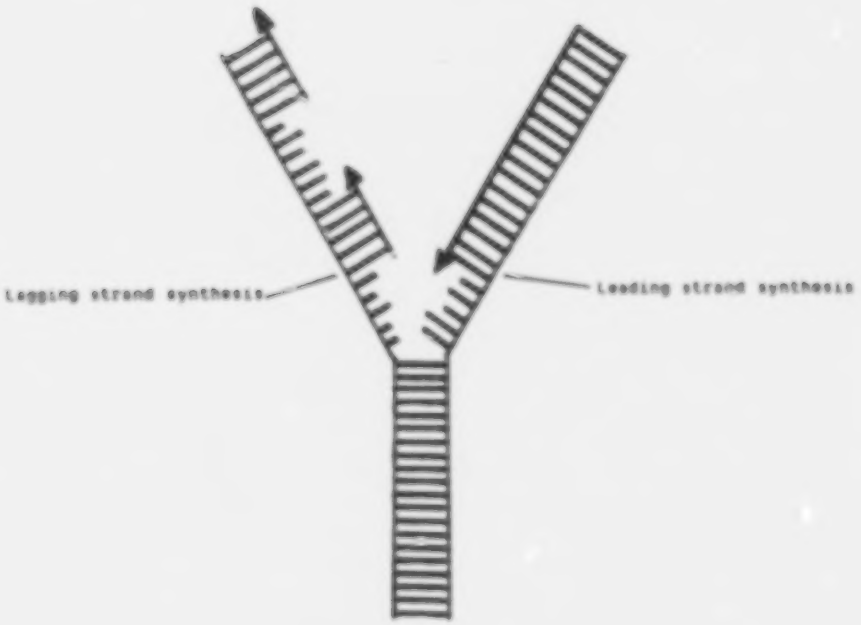

Figure 8. DNA replication fork.

1st position (5' end) ↓	2nd position U	C	A	G	3rd position (3' end) ↓
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Figure 9. The genetic code.

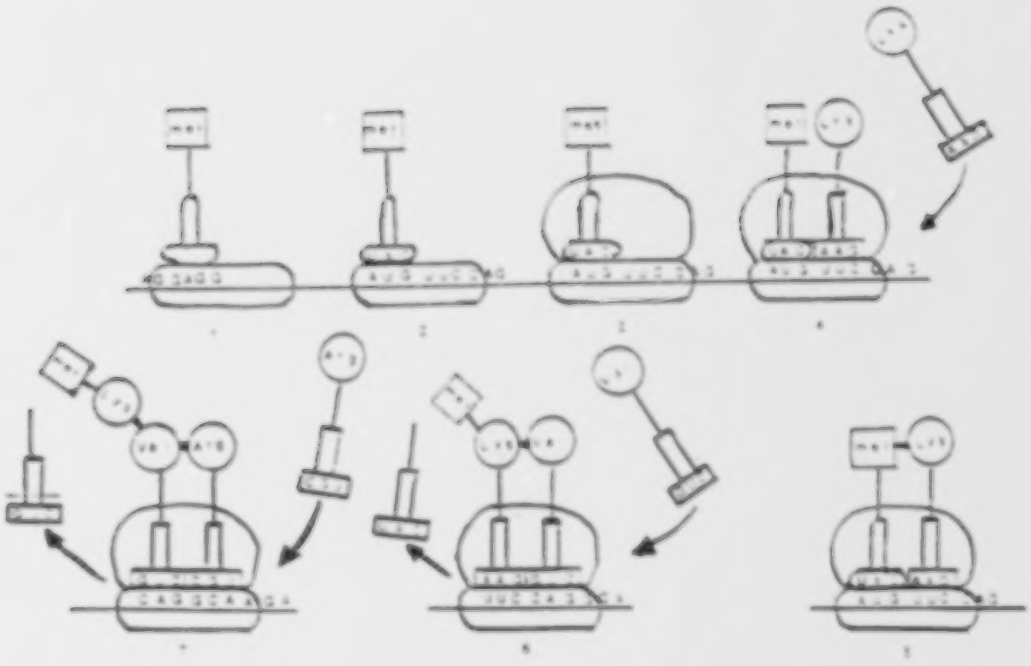

Figure 10. Translation of the genetic code into protein.

1. Small ribosomal unit with initiator tRNA binds to mRNA.
2. Binding of initiator + RNA to start condon AUG.
3. Large ribosomal sub-unit binds to small ribosomal sub-unit.
4. tRNA carrying amino acid binds to ribosome.
5. First peptide bond formed.
6. Ribosome shifts one codon to right and second tRNA + amino acid binds and bonds.
7. Ribosome shifts one codon to right again and third tRNA + amino acid binds and bonds.

The Use of Microbes in Biotechnology

Several features of microorganisms make them the most exploited group of organisms in biotechnology to date. Among those features are their ease of culture, their rapid growth cycles, the very diverse types of metabolism found--which give a wide range of useful commercial products, and the ease with which the relatively simple genome can be manipulated to enhance their usefulness. Table 1 shows that a wide range of bacteria, yeasts, and filamentous fungi already is in use to produce food products, industrial chemicals, hormones and antibiotics. There is, however, a constant search for organisms that produce new products, and biologists are sampling all kinds of habitats for new microorganisms. The more extreme the environment, the more likely it is that the microorganisms found there will differ in their cell chemistry from organisms from a more equable habitat. Thus, the deserts of tropical and polar regions, mineral-bearing sands and schists, and tar and oil deposits will be sampled. Hot springs and alkali lakes may provide suitable habitats for microorganisms that produce thermo- and alkali- stable enzymes respectively.

Any microorganism used in biotechnology should be easy to culture and should be non-pathogenic. It is useful, but not a requirement, that the microorganisms in use have a high product yield, because the yield of a high-value product can probably be increased economically by strain and culture improvements in the laboratory. Various mechanisms to improve product yield are discussed below.

1. *Selective Isolation.*

The identification and isolation of interesting microorganisms from a population of wild or laboratory-induced microorganisms can be expensive and time consuming. In the past, a large number of isolates was cultured and screened. This was cost effective at first, but as the more common species were isolated and exploited, this method was abandoned for more rational approaches.

Habitat samples may first be heat treated to remove the more common vegetative cell flora and thus isolate spore-forming species. If one seeks an organism that will degrade a particular molecule, the habitat may be enriched with the substance so that the organisms of interest will predominate. Alternatively, a mixed sample may be cultured in an enriched medium so that again the microorganisms that can make use of the enriching substance will predominate after repeated batch cultures. Antibiotics can be used to suppress molds, yeasts, and certain species of bacteria to allow the rarer forms to grow. One can sometimes identify a microorganism through a product that is not of particular interest. For example, the activity of proteolytic enzymes, which is easily detected, may be associated with the production of antibiotics.

Table 1. Some common industrial enzymes, their sources, and uses.

Enzyme	Source	Principal uses
α-Amylase	*Aspergillus oryzae*	Starch hydrolysis for sugar
	Bacillus amyloliquefaciens	syrups, brewing
	Bacillus licheniformis	textiles and paper
Cellulase	*Aspergillus sp.*	fruit and vegetable
	Trichoderma rusei	processing
	Penicillium sp.	
β-Glucanase	*Bacillus subtilis*	β-Glucan hydrolysis
	Aspergillus niger	brewing
Glucoamylase	*Aspergillus niger*	Glucose syrup production
	Rhizopus sp.	from liquified starch
Glucose isomerase	*Actinoplanes missouriensis*	Isomerization of glucose
	Streptomyces sp.	into high fructose syrups
Lactase	*Saccharomyces sp.*	Hydrolysis of lactose
	Kluyveromyces marxianus	in milk and whey
Lipase	*Aspergillus sp.*	cheese and butter flavor
	Mucor sp.	modification. Fat and oil
	Rhizopus sp.	processing
Pectinase	*Aspergillus niger*	extraction and clarification of fruit juices
Penicillin amidase	*Bacillus megaterium*	synthesis of
	Escherichia coli	6-aminopenicillanic acid for manufacture of semisynthetic antibiotics
Protease (alkaline)	*Bacillus licheniformis*	detergent and leather industries
Protease (neutral)	*Bacillus amyloliquefaciens*	baking and brewing
Protease (acid)	*Endothea parasitica*	cheese manufacture
	Mucor miehei	
Pullulanase	*Klebsiella pneumoniae*	debranching starch in sugar
	Bacillus acidopullulyticus	syrup manufacture

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2. *Strain Improvement.*

Once an organism that makes a potentially useful product has been identified, one must assess its likely cost of production and its market value before deciding whether it is suitable for strain-improvement procedures. If the product has some new commercial or medical applications that will provide a breakthrough, it will be developed even if it looks as though it will be expensive to produce. One can improve the productivity of an organism--and thereby production-unit costs--by manipulating protein synthesis, by interfering with the genome, or by using specially designed and cheaper media.

A. Control of transcription

The transcription of nucleotide sequences is controlled by several factors, some of which can be modified to increase the efficiency of mRNA production for protein synthesis. The features of transcription in procaryotes and eucaryotes are similar in principle, but differ in detail. Because the details are more complicated in eucaryotes, the process in procaryotes is described here to illustrate how transcription may be modified.

The essential feature of transcription, as described earlier, is the binding of an RNA polymerase molecule to the double-stranded DNA upstream of the gene to be transcribed. The enzyme is assisted in this binding by small protein subunits, the sigma (σ) initiation factors. These sigma factors bind to the RNA polymerase and recognize two sets of DNA hexanucleotides, which are called the consensus promoter sequences (figure 11). These are situated -10 and -35 nucleotides upstream from the DNA "start" signal. In addition to serving as the binding site for the RNA polymerase, these promoter sequences, by reason of their asymmetry, also determine which way the polymerase will pass along the DNA double strand during transcription. Because the polymerase synthesizes RNA only when it travels in the 5'-->3' direction, it also determines which of the two DNA strands will serve as the template. Transcription also is controlled by proteins attached to the operator, a nucleotide sequence between the promoter and the start signal, or upstream from the promoter. These control proteins are switched on or off according to the intracellular levels of specific metabolites, which will vary according to the cell's food sources.

One of the first of these regulator proteins whose mode of action was unravelled is the lactose repressor protein of *Escherichia coli*. The lac repressor, bound to the operator (figure 12) prevents the RNA polymerase from starting RNA synthesis at the promoter. This in turn blocks transcription of the downstream region of the DNA in which three genes (the lac operon) code for three enzymes that are involved in lactose metabolism. The lac repressor protein controls the production of these enzymes according to the needs of the cell.

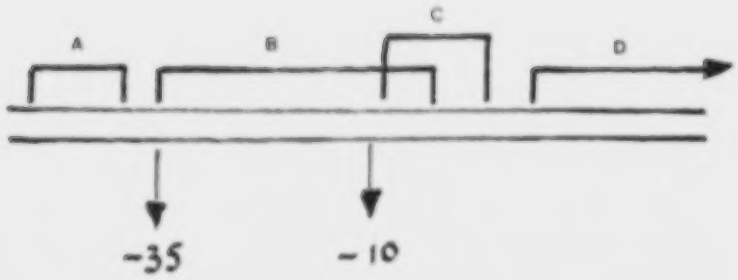

Figure 11. Transcription control sites.

A. Catatabolite activator protein (CAP) binding site.
B. Promoter - two hexanucleotide sequences at -35 and -10
C. Operator sequences
D. Messenger RNA gene sequences

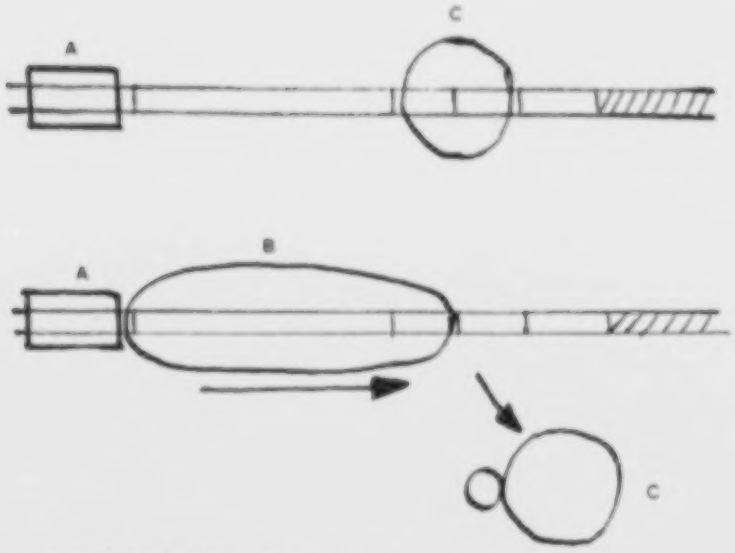

Figure 12. De-repression of the lac operon.

A. Catabolite activator protein (CAP)
B. Repressor protein bound to operator site
C. RNA polymerase binds in absence of repressor protein and transcription begins.
D. Repressor protein removed by addition of allolactose

When lactose is present in the medium, a small sugar molecule called allolactose is formed in the cell. This molecule binds to the lac repressor protein, and by altering its shape releases it from its binding site on the DNA (de-repression). This can allow transcription to proceed, and lactose to be used as a substrate. In the case of the lac repressor (and many others), however, this de-repression is not sufficient if glucose (the preferred metabolite) is also present in the medium.

The lac promoter does not exactly match the *E. coli* promoter consensus sequences and binds the RNA polymerase so weakly to the consensus sequence that it is not able to transcribe the DNA. In the absence of glucose, a catabolite activator protein (CAP), bound to the DNA just upstream from the promoter, improves the binding of the RNA polymerase, and transcription proceeds.

The binding of the CAP to the DNA is governed in turn by cyclic adenosine monophosphate (cAMP). In the absence of glucose, the preferred carbon source, the *E. coli* cell divides more slowly, and the levels of cAMP increase. In the presence of high cAMP levels, the CAP molecule can bind to the DNA binding sites. In turn, the CAP molecule bound to the DNA produces conformational changes in the RNA polymerase that lead to its binding strongly to the promoter. If lactose and allolactose are present, the repression molecule is removed and the transcription of the lac operon proceeds.

This system ensures that the cell does not produce enzymes until they are required. When the preferred sugar is present, the synthesis of other sugar-metabolizing enzymes is suppressed by the inability of CAP to bind to the DNA because of the low level of cAMP in an actively dividing cell.

There are many other examples of this type of gene-transcription control in bacteria and other cells. The mechanisms can vary, however. For example, whereas in the lac repressor protein the signal molecule (allolactose) decreases the affinity of the repressor molecule for the DNA and thus *turns on* a gene or operon, in some cases the signal molecule increases the binding and *turns off* a gene. Thus, a rising level of the amino acid tryptophan being synthesized in a cell leads to the tryptophan operon being turned off by increasing the binding of the tryptophan-repressor protein.

In microbe-improvement programs, catabolite repression may have to be modified to produce a desired industrial enzyme. This can be done by avoiding the use of repressing carbon sources in the medium, such as glucose in the case of the lac operon example. Other methods include limiting the growth rate of a culture, and thereby keeping cAMP levels high, by the use of slowly metabolizable analogues of the repressing substrate, or by feeding the usual substrate slowly. A further

possibility is to improve promoter binding by a mutation that will minimize the role of a catabolite activator protein.

B. Control of translation

Translation does not have as many control mechanisms in procaryotes as does transcription, and thus it offers less opportunity for modification. Translation requires the binding of an initiation factor to the small ribosomal subunit to enable it to bind to the consensus sequence (AGGAGGU) on the mRNA and form an initiation complex. The large ribosomal subunit now binds to the complex, and translation begins, as shown in figure 10.

There seems to be considerable variation in the relative efficiency of the mRNA ribosomal binding site of different genes, and this variation offers the possibility of modification to improve the efficiency of translation. A further possibility is to improve the stability of procaryotic mRNA. Most procaryotic mRNAs have a very short half-life--about four minutes; an extension of this half-life would improve efficiency.

Translation in eucaryotes is more complex and less well understood. It does seem, however, to have more control points, which may make translational control more accessible for modification.

C. Culture improvement

In addition to methods for the restriction of catabolite repression, one can improve the overall productive efficiency of microorganisms by careful monitoring and control of the culture method. In addition to the provision of an optimum physical environment for a particular organism (pH, temperature, and optimum medium-organism contact), the efficiency can be improved by supplementation of essential growth constituents, maintenance of essential inducer concentrations, and control of end-product accumulation. The foregoing discussion showed that enzyme production for lactose degradation can be controlled by induction. Product accumulation in the culture medium can lead to feed-back repression of enzyme biosynthesis and can be avoided by ensuring that products do not accumulate in the medium (as in continuous culture methods), or by the selection of mutants that are not repressed by end products.

D. Manipulation of the genome

A variety of methods is available for the manipulation of the genome to improve the productivity of a microorganism. Sexual hybridization is a natural way for organisms to exchange genetic material and produce new gene combinations

from the reorganization of the genes during meiosis and the process of hybridization.

Haploid nuclei from opposite mating types with different genomes are brought together in one cell. The diploid nucleus so produced undergoes meiosis that further reorganizes the genetic elements. This method has been used to improve strains of yeast in the baking, brewing and sugar-fermentation industries, but is has limited application because few industrially important microorganisms show sexual hybridization.

This limitation can be overcome by artificial cell-fusion techniques or by using viral and plasmid vectors to carry part of the genome of one cell into another. A further method is to bring about changes in the genome by mutagenesis. A variety of chemical mutagens is available (nitrogen mustard, 5-bromouracil, and methylmethane sulphonate, for example), as well as UV and ionizing radiations. These mutagens have a double effect on the genome: they cause damage to the DNA sequences and they also affect the DNA repair processes that would otherwise make good much of the damage. Mutagenesis, followed by the selection of improved strains, has been the main approach to increased productivity so far.

Protoplast fusion allows artificial mixing of hereditary material in species that do not show hybridization. The cells of bacteria, fungi, and plants have an external cell wall of complex polysaccharides that prevents cell fusion. If this cell wall is dissolved by enzymes, a naked protoplast is exposed. The protoplast cell membranes of different species have very similar molecular structures and can be fused, with the assistance of polyethylene glycol and Ca^{++}. Moreover, the fused protoplasts in many instances regenerate a new cell wall and resume normal growth.

In this way haploid nuclei of different strains are brought together in one cell, where they fuse to form diploid nuclei, which eventually undergo meiosis and further reorganizations of the hereditary material. Protoplast fusion produces new arrangements of the whole genome, which is particularly useful where large numbers of genes are involved in the synthesis of products such as antibiotics.

The technique developed for the fusion of protoplasts has also been used for cell fusion--the direct fusion of animal cells. One of the most spectacular and profitable uses of cell fusion is the production of monoclonal antibodies.

The traditional method for producing antibodies for health care is to inject a foreign protein or attenuated microbe into a vertebrate, where the plasma cells develop antibodies to the foreign, antigenic protein. Antibodies produced in this way will result in antisera containing a very heterogenous collection of antibodies. An antigenic protein will have many antigenic sites (epitopes), so the antibodies produced will be derived from many cells with immunoglobulins to all the antigenic

epitopes. Each B-lymphocyte produces an antibody to a specific epitope; if, therefore, a particular lymphocyte could be isolated and cloned it would be possible to produce a monoclonal antibody, which would be highly target selective. Unfortunately, B-lymphocytes only live for a short time *in vitro*, ruling out direct cloning. This difficulty has been overcome by fusing the lymphocytes with malignant, rapidly dividing, myeloma cells, which grow indefinitely in culture. The two properties of the fused cells--antibody production and continuous proliferation--are expressed.

An outline of the procedure is as follows: Activated B-cells of a mouse immunized with a specific antigen are obtained from the spleen. The B-cells are fused with the myeloma cells by incubation in the presence of polyethylene glycol. About one in a thousand myeloma cells fuse with a B-cell. The unused B-lymphocytes die in culture, and the myeloma cells can be eliminated by using an anti-metabolite of the strain of myeloma cells used. The hybrid cells (hybridomas) are diluted and screened for antibody. Positive clones are subcloned and developed into mass cultures. Cells producing the desired monoclonal antibody can be cultured in a mouse ascites tumor, where in the few milliliters of fluid, 5-20 mg/ml of specific antibody can be obtained. Large scale production is now possible by growing the hybridomas in air-lift fermenters, where yields of some 50 mg/ml are possible.

Monoclonal antibodies, because of their high specificity, accuracy and ease of use are among the fastest-growing segment of biotechnology. They already have widespread use as a diagnostic and therapeutic tool. Among the current applications of monoclonal antibodies are:

1. identification of microbial pathogens;
2. detection of drugs in a person's blood;
3. detection of hormones;
4. localization of tumors with specific antibodies;
5. targeting of cancer cells with immunotoxins; and
6. improvement of the acceptance of organ transplants by injection of antibodies against certain antigens.

E. Recombinant DNA technology

This provides another method of introducing nucleotide sequences into a host organism for the production of useful protein products for agriculture, pharmaceutical, food, and other industries.

Unlike the other methods described above, recombinant DNA technology permits isolation, purification, and amplification of selected gene sequences. Viral or plasmid vectors can introduce the selected sequences into a producer organism,

where they become a permanent, functional part of the host genome. The "scissors" that are used to cut the donor DNA molecule into fragments are enzymes made by bacteria to protect themselves against foreign DNA carried into the cell by bacterial viruses. About one hundred of these restriction enzymes are now available commercially.

Each restriction enzyme recognizes a particular set of six-to-eight nucleotides and cleaves the double-stranded DNA at these sites to produce a number of restriction fragments. Many restriction enzymes make staggered cuts, leaving single-stranded ends on the fragments, as illustrated in figure 13. The unpaired nucleotide bases on these single-stranded ends will readily link with complementary single-stranded ends, in the presence of DNA ligase. Where the restriction enzyme makes a straight cut and leaves a blunt, two stranded end, they can be linked with some difficulty by DNA ligases, or by the use of a chemically synthesized "nucleotide-linker" molecule.

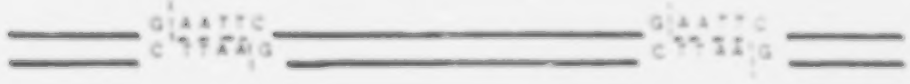

Figure 13. The production of DNA restriction fragments by the restriction enzyme EcoR1; the procedure produces "sticky" ends.

DNA fragments produced in this way can be introduced into another cell using plasmids or bacterial viruses (phage) as vectors. Plasmids are small DNA or RNA replicating, extra-chromosomal units that are formed in all bacterial, yeast, plant, and animal cells. They are small, double-stranded, circular molecules that can be separated fairly easily from other cell contents by centrifugation of crushed cells. Viruses differ from plasmids in that their nucleic acid has a protective coat or capsid, which enables them to exist outside a cell.

To prepare a plasmid for use as a vector, its DNA is cut with the same restriction enzyme used to produce the donor DNA fragments. A wide range of plasmid vectors is available. The one selected should have a single site for the enzyme to be used, and it should have genes for antibiotic resistance (which plasmids commonly have) to assist in the identification of the transformed hosts.

The restriction enzyme opens up the circular plasmid. When DNA donor fragments are added (in the presence of DNA ligase), the fragments can be taken up into the complementary site of the vector. A very mixed population of molecules is obtained, but a few plasmids will become a hybrid recombinant with a new, inserted DNA fragment (figure 14).

Figure 14. Production of a plasmid vector using rDNA technology.
A. Plasmid spliced by ECORI restriction nuclease.
B. Restriction fragment inserted into opened plasmid vector and annealed by DNA ligase.

The hybrid vector is then introduced into a host cell. Various techniques are available for making the host cells permeable to the plasmids. When *E. coli* is used, the plasmids and bacterial cells are mixed in cold calcium chloride and the mixture warmed to 37°C for five minutes. During this time, the plasmids are taken up into the bacterial cell. The cells are then transferred to a broth medium, incubated for a time and examined for the product of the DNA insert or some phenotypic character associated with it. Those bacteria carrying the desired hybrid molecule are purified by isolation and cultures. The division of the host cells and the replication of the hybrid plasmids within them produce multiple copies of the selected gene. Provided the selected plasmid vector has efficient promoter sequences, gene cloning by this technique will increase the productivity of the host organism.

Fragments of DNA derived from a cellular genome by a restriction enzyme will produce a very large number of fragments--as many as a million, for example, from a mammalian genome. There will therefore be a very large number of cell colonies following transfection, each colony produced from a single cell. This makes identification and selection of the clone carrying a specific DNA fragment derived form genomic DNA difficult, time consuming, and expensive.

Another option is to use DNA fragments made by copying a selected and purified mRNA from a cell. A viral enzyme, reverse transcriptase, enables a complementary, single-stranded DNA copy (cDNA) to be made from the mRNA molecule, a reverse of the usual transcription process. The single-stranded DNA can be converted into a double-stranded fragment by DNA polymerase. This double-stranded fragment can be introduced into bacterial cells by plasmid or viral vectors, as described above, and cloned. This method gives a very much more uniform set of cell colonies, so the process of identification of transformed cells is greatly simplified.

Because identification is expensive and time consuming, the biotechnology industry keeps the methods it uses as closely guarded secrets. Among the methods known to be employed are the use of antibodies to detect protein products, and the use of plasmids containing genes for antibiotic resistance, which give the host cell resistance to specific antibiotics. One such plasmid has resistant genes to ampicillin and tetracycline. By inserting the foreign DNA fragment into the site of the gene for tetracycline, transformed cells that are ampicillin resistant but tetracycline sensitive will contain the hybrid plasmid.

F. Determination of nucleotide sequences

Fragments of DNA produced by restriction enzymes can be separated rapidly according to their size by gel electrophoresis. An electric potential is applied across a polyacrylamide or agarose gel. The negative charge on the fragments leads to their migrating through the gel matrix towards the positive end of the electric field. The relative speed with which they move will depend on the size of the fragment, the smaller ones moving faster than the larger ones in a given time. Incorporation of fluorescent strains or radioactive phosphate into the DNA molecule allows the positions of the fragments to be made visible and recorded.

Techniques are available for the rapid determination of a nucleotide sequence in any purified fragment of DNA. These techniques are now so accurate and rapid that the easiest way to determine the amino-acid composition of a protein is to determine the nucleotide sequence of its gene. The mRNA of the gene is converted into cDNA, its sequence determined and the genetic code used to interpret the nucleotide triplets into the amino-acid sequence.

One sequencing method uses a chemical to cleave the DNA at specific nucleotide sites. Four samples of a restriction fragment, labelled at one 5' end with ^{32}P-phosphate, are subjected to treatment by chemicals that preferentially cleave DNA at nucleotide A in the first sample, at G in the second, C in the third, and T in the fourth. The fragments from the four samples are simultaneously separated

by gel electrophoresis, giving a pattern of radioactive bands from which the DNA nucleotide sequence can be read. Figure 15 illustrates this process.

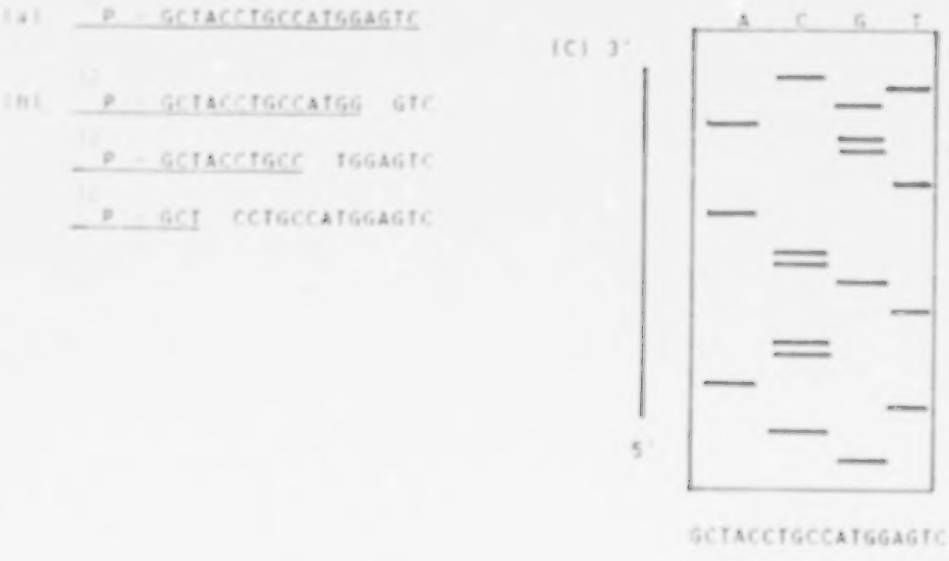

Figure 15. Determination of DNA sequences.

- **(a) Single-stranded DNA restriction fragment labelled with ^{32}P.**
- **(b) Fragment treated to produce a family of sub-fragments cleaved at a particular nucleotide (in this example the A nucleotide).**
- **(c) Four samples of restriction fragments cleaved at A, or C, or G, or T. Electrophoresis of the sub-fragments provides a pattern of radio-active bands from which the nucleotide sequence can be read.**

G. Nucleic acid hybridization

Nucleic acid hybridization offers another method for the identification of specific nucleotide sequences. When double-stranded nucleic acid is heated to 100°C in aqueous solution, the complementary base pairs break down and the double helix forms two single strands. If complementary single strands are maintained at 65°C for a long period, the two strands will reform a double helix by the process of hybridization. Such hybridization can take place between two DNA or RNA strands, and mixed DNA/RNA strands, provided they have complementary nucleotide sequences.

Nucleic-acid hybridization is now widely used to detect the presence and concentration of a particular DNA or RNA sequence. A DNA fragment with the specific nucleotide sequence of interest (a DNA probe) is produced by cloning or chemical synthesis, labelled with ^{32}P and added to an aqueous mixture of the sample being studied. The progress of its hybridization can be followed by measuring the radioactivity taken up by the sample. The rate at which hybridization takes place is a measure of the concentration of the DNA or RNA sequences, because hybridization also depends on the chance collisions of the radioactively labelled DNA probe with the unlabelled molecules.

DNA probes are extremely sensitive and selective. When used in hybridization with mRNAs purified from a cell, a DNA probe containing a selected gene sequence will show the presence or absence of the gene in the cell, and the number of copies present. DNA hybridization can allow one to follow changes in gene activity during development, can detect genetic disorders in a fetus, and is used increasingly in forensic science.

When combined with gel electrophoresis, DNA probes can be used to detect nucleic acid fragments. A convenient way to carry this out is by a technique known as blotting. A purified DNA sample to be examined is broken into fragments with a restriction enzyme, and these are separated according to their size by gel electrophoresis. A replica of the gel is made, transferring the fractionated DNA fragments onto nitrocellulose or nylon paper by blotting (Figure 16) to make the fragments accessible to the probe. A ^{32}P DNA probe with the specific nucleotide sequences of interest is incubated with the blot to hybridize any complementary molecules. The location of the probe on the paper is revealed by autoradiography. This process is known as Southern blotting. A similar technique using DNA probes to identify RNA sequences is known as Northern blotting. Both techniques make it possible to detect accurately the presence of a specific sequence in normal genes, and defects (deletions and insertions) in mutants.

A particularly interesting development of this technique, known as DNA fingerprinting, is proving to be an invaluable tool in paternity and forensic cases. It requires only a very small sample of a DNA source--blood, semen, or body tissue. The DNA is extracted from the sample, purified, cut into fragments by a restriction enzyme, and separated into bands by gel electrophoresis. The gel is blotted with nitrocellulose paper and the paper incubated with a radioactive DNA probe prepared from a sample of blood from the suspect. A comparison of the autoradiograph bands of the two reveals any common DNA fragment bands, as illustrated in figure 17.

DNA fingerprinting allows the paternity of a child to be determined with a very much higher degree of certainty than did the previous tests, which were based

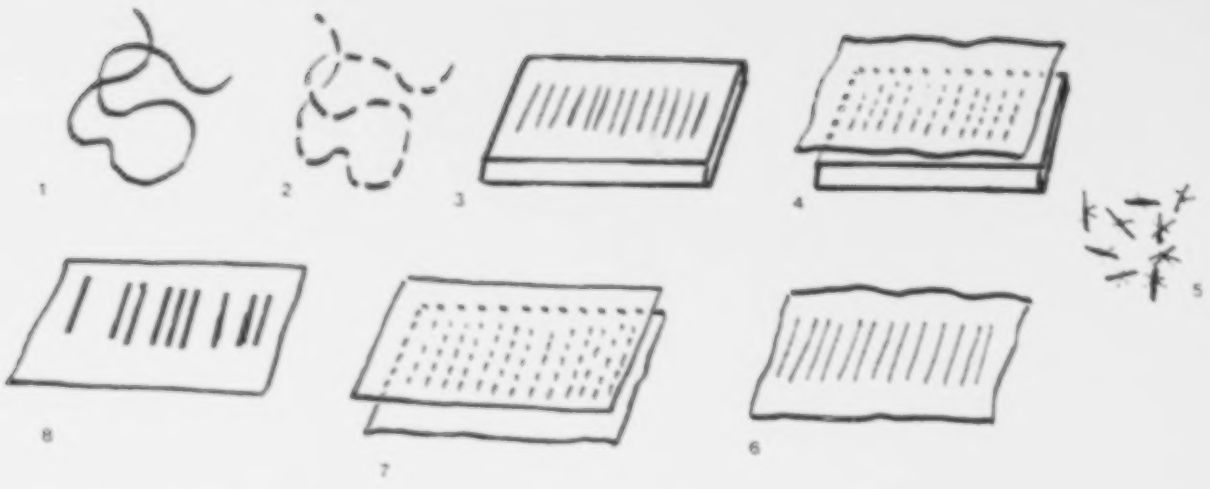

Figure 16. Southern blotting
1. Purified DNA Sample.
2. DNA cut into fragments with restriction enzyme.
3. DNA fragments separated into bands by electrophoresis in agarose gel.
4. Band pattern is transferred to nylon paper - Southern blotting.
5. Radio-active DNA probe.
6. Southern blot bathed with radio-active probe which binds to specific DNA sequences on the nylon paper.
7. X-ray film placed in contact with nylon paper to detect radio-active pattern.
8. X-ray film developed to reveal the pattern of bands identified by the probe.

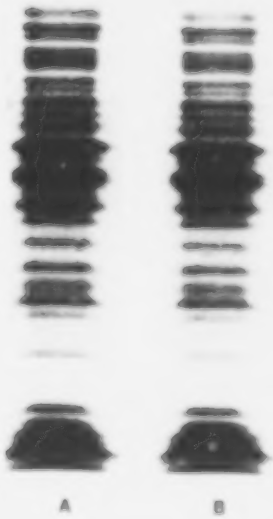

Figure 17. DNA Fingerprinting.
A. Blood sample from victim
B. Blood sample from suspect

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on blood-group antigens. The technique is proving to be invaluable in cases of homicide, rape, or robbery with violence. The criminal can readily and reliably be identified from amongst a group of suspects from a very small sample left at the scene of the crime. The forensics community has not yet established standards for DNA fingerprinting, and variations in test results have caused some jurists to disallow the results as evidence. These problems should be resolved by the development of industry-wide guidelines for testing and reporting.

Fermentation Systems

The earliest fermentations were carried out in shallow depressions in the ground, in ponds or ditches, using natural sources of enzymes, and fruit or other plant foods as fermentation media. In many parts of the world, such simple systems are still used for many important processes, such as the treatment of waste and the retting of cotton. From such simple, open fermenters, it was a short step to vessels of wood or ceramics, and on to the vessels of metal in use today. Fermentation in shallow, open containers is aerobic. By deepening the vessel and providing a cover, fermentation can be made to proceed anaerobically. Such tank fermenters have been used for many years to make beer, wine, vinegar, yogurt, and many other traditional drinks and foods. Tank fermenters provide a means of controlling the availability of oxygen, the temperature, the fermentation medium, and the microorganisms to be used. Fermentation today is big business, and fermenters are carefully designed, using the expertise of microbiologists and chemical and process-control engineers to exploit the biological activity of the microorganisms or plant and animal cells and to obtain maximum output and purity of the product at minimum cost.

1. *Types of Fermenters.*

There are three types of present-day fermenters for microorganisms: (A) stirred and aerated, (B) air-lift, and (C) solid-substrate. These may be operated as batch processes or continuous fermentation. Continuous fermentation has not, however, been generally successful on the industrial scale.

A. Stirred-tank fermenters

To provide the special conditions required by most industrial fermentation today, the fermenter is a closed vessel, in which the temperature, pH, and aeration can be controlled, along with the stability, viscosity, and morphology of the medium. The fermenter and its ancillary equipment must be easy to clean and sterilize between batches, and provision must be made for sterile inoculation, sampling, and control of the fermentation.

The vessels are made of stainless steel. The joints are butt-welded and

carefully polished to avoid ledges or grooves in which medium and microorganisms may lodge, causing problems in cleaning and sterilization. A typical stirred-tank fermenter is shown in figure 18. Most fermentations operate aerobically.

Air sterilized by filtration is pumped in at the bottom of the culture medium through a sparger (diffuser). The design of the sparger is to some extent influenced by the microorganisms being used. Spargers with fine holes may become blocked by the growth of some microorganisms. In culture media of low viscosity, the stream of air bubbles provides sufficient stirring and agitation of the culture to keep it dispersed and well mixed. Paddles mounted on a shaft driven by a motor are usually necessary to provide the right amount of agitation, however. The shaft bearing in the wall of the fermenter must be designed to prevent access of foreign microorganisms. This is often achieved by designing a small chamber in the bearing through which steam is passed. To prevent simple swirling of the culture medium, baffles are mounted vertically on the inner wall of the fermenter.

Agitation of the medium increases the solution of the oxygen in the medium, prevents the microorganisms from forming aggregates that would impede exchange between the cells and the medium, and increases the efficiency of heat transfer from the fermenter to the cooling system. Efficient agitation and aeration depend upon the shape of the fermenter, the design of the agitation system, and the physical properties of the medium.

Modern fermenters are cylindrical in shape. Those that use mechanical agitation have a depth/width ratio of 3 to 4 : 1 to provide a long pathway through the culture medium and to provide better agitation.

The volume of the fermenter will depend on the nature and range of the fermentation being carried out. Small industrial fermenters (40-200,000 liters), used with batch techniques, have the advantage of greater flexibility. They can be used to produce smaller quantities of the product, which may only be required intermittently by market outlets. They enable the producer to switch their use to products in demand. Changing the agitation, aeration, temperature control, and pH to suit each fermentation system is relatively quick and easy with small fermenters. Furthermore, should contamination occur, the damage is restricted to small volumes of culture medium, which may well contain expensive ingredients.

The shape, number, and speed of rotation of the paddles all affect the agitation of the medium. The shape and number of the paddles, together with the optimum tip speed, are worked out in pilot plants for each type of medium and microorganism proposed for use. Most industrial fermenters have three or four sets of paddles arranged along the length of the motor shaft, spaced roughly equal to the diameter of the cylinder.

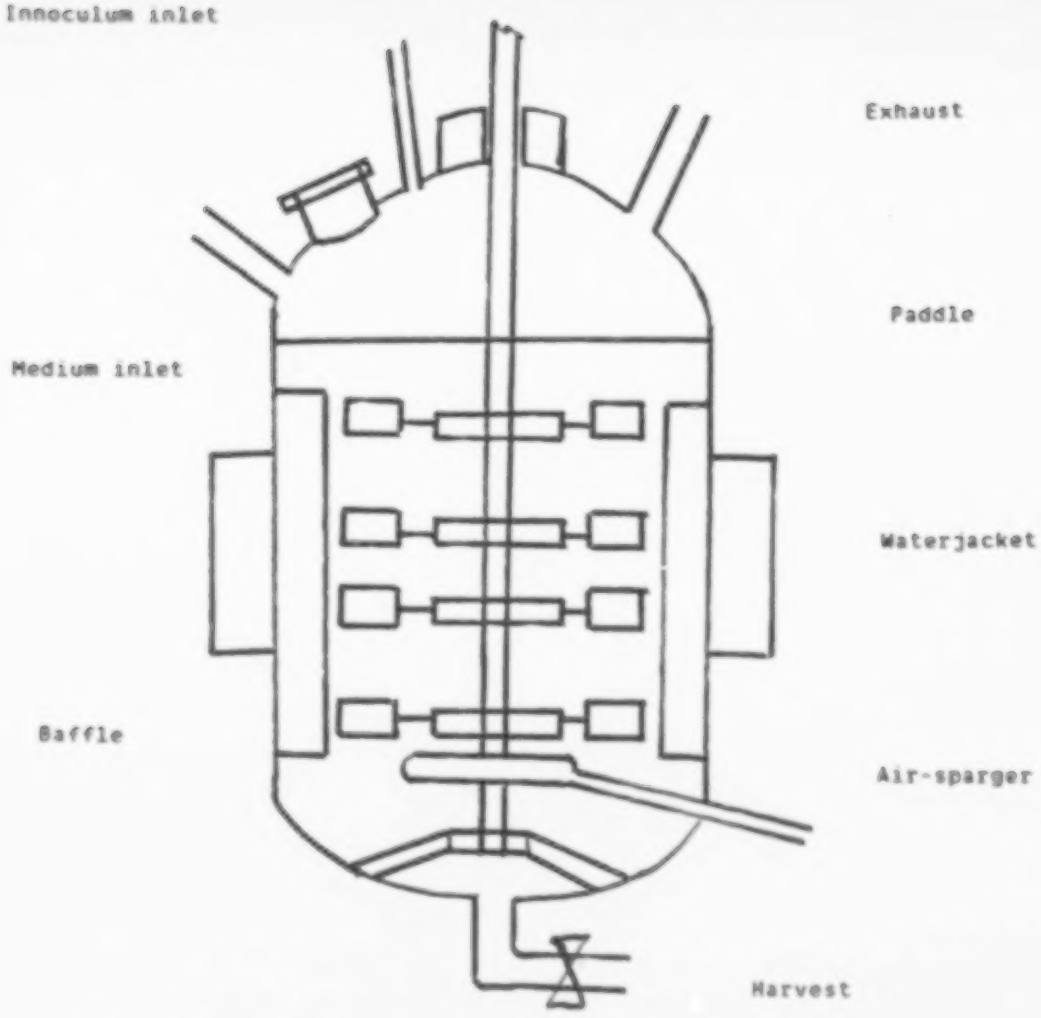

Figure 18. Stirred-tank fermenter

The viscosity of the medium and the flocculating tendency of the microorganism during culture will determine the optimum characteristics. The efficient dispersal of the microorganisms and the air bubbles in the medium must be balanced against the energy costs of rotating the motor shaft, which can be high.

B. Air-lift fermenters

The paddles of a stirred-tank fermenter can have a damaging effect on the cells. They also increase the difficulty and expense of cleaning and sterilizing the fermenter. Special precautions must be taken to seal the bearing of the paddle shaft to prevent infection of the reactor from the outside. As indicated above, a stirred-tank fermenter requires a considerable amount of energy, particularly in large industrial fermenters.

These problems can be overcome or made less severe by using sterilized air bubbles introduced into the base of the fermenter to mix the medium and the microbes, as in the Tower reactor and similar designs (figure 19). This method of agitation is only effective with the more fluid media in tall, narrow fermenters. This geometry improves the solution of oxygen into the medium, partly because of the higher pressure of gas at the base of the tall column, and partly through the longer path and better agitation. The sterile air is introduced through spargers with fine holes to create bubbles of small diameter that will have a large interfacial area and rise more slowly through the medium. The actively metabolizing microbial cells produce a certain amount of heat. A cooking jacket or a cooling coil immersed in the medium is fitted to provide a means of controlling the temperature. Cooling coils add to the difficulty of cleaning the vessel, but they are necessary in the larger fermenters (above 1,000 liters), as jackets do not have sufficient area of contact with the fermenter wall to remove the excess heat.

Another form of air-lift fermenter is the loop reactor, in which the liquid medium is forced to circulate in one direction through the reactor. The circulation of liquid, which is accomplished by internal baffles or an external recirculation pipe, is begun by injecting air at the base of the column. Once the liquid movement is underway, air is injected into the downward circulation. This has the advantage that as the air is carried downwards, it comes under increasing pressure, enhancing its solution. In the upward-moving column, the air bubbles expand, maintaining the movement of the liquid medium after the initial injection of air at the base of the column is switched off. The rapid circulation of the medium simplifies temperature control. It is not usually necessary to have cooling coils or jackets in air-lift fermenters.

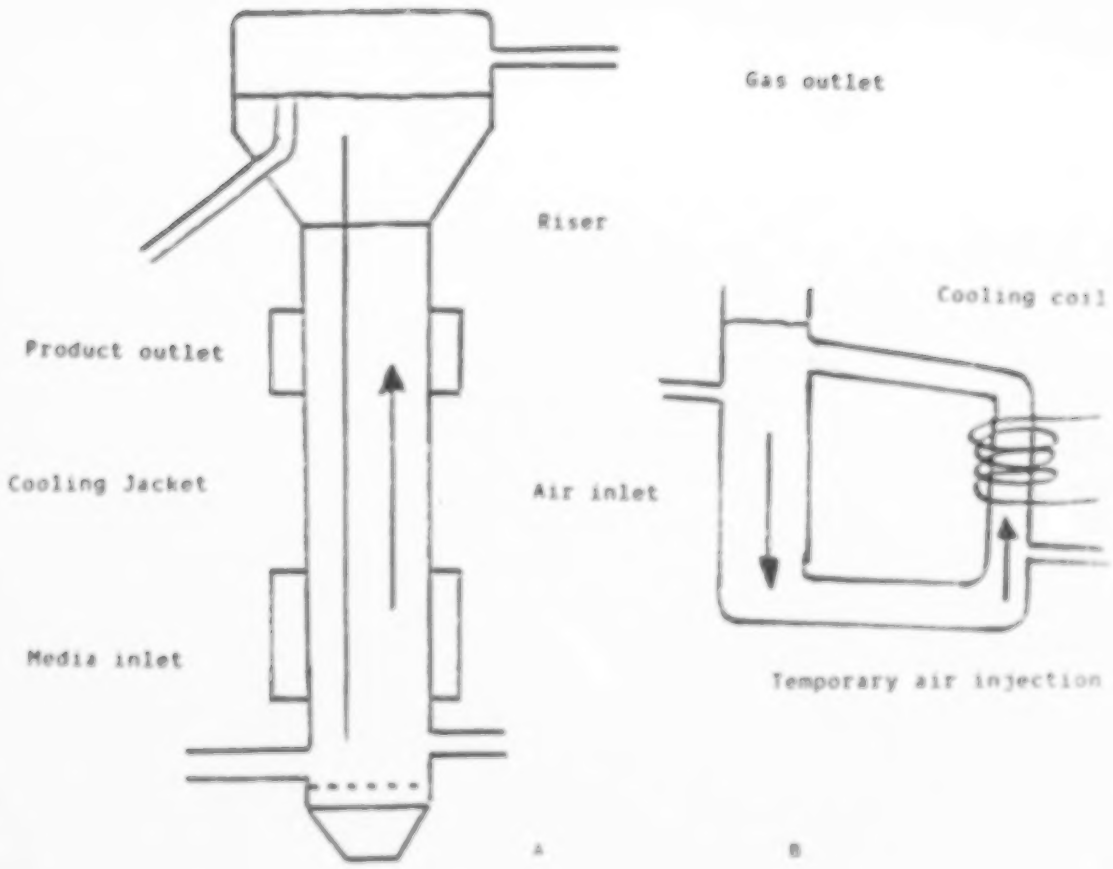

Figure 19. Air-lift fermenters.
A. Tower reactor.
B. External loop reactor.

C. Solid-substrate fermenters

Solid-substrate fermentation has been used for very many years in the Far East and many other regions with warm climates. In many instances the equipment required is simple and inexpensive. It is labor intensive, but the cost of labor is insignificant in many of the countries in which it has been traditionally used, and its use has served a social function.

Solid-substrate fermentation, as its name applies, is carried out on the surface of insoluble biological materials, such as cereals, rice, seeds, agricultural and horticultural waste, wood, and straw. It occurs in the near absence of water. Many of the substrates require some form of pretreatment. This may be mechanical treatment to reduce the particle size and thus provide a large area of contact with the microorganism employed, or it may be chemical, to hydrolyze partially the organic polymers in the substrate and thus bring them to a chemical structure suitable for microbial fermentation.

Particle size may need to be adjusted carefully to provide a good sponge-like structure, with good air spaces between the particles. This is essential because most solid-substrate fermentations are aerobic, and transport of oxygen within the medium can become a critical factor in the successful growth of the fermenting organism and formation of the product. Oxygen and CO_2 levels must be monitored within the substrate, and, if necessary, aeration and agitation used to maintain satisfactory levels.

Aeration and agitation also are used to control the temperature of the solid substrate. Heat generated by microbial activity is much greater per unit volume than in liquid fermentations and is less easily transferred to the surfaces of the container. In large industrial operations, the temperature and moisture content of the circulating air is used to assist heat transfer.

The type of bioreactor used with solid substrates depends upon the frequency with which agitation and forced aeration are required. Fermentations that require regular agitation as well as aeration are carried out in racks of shallow trays. This type of reactor may be simple, but it is labor intensive. Where occasional agitation is required, rotating drums may be used. In more sophisticated equipment, optimum conditions for growth and production are provided by placing the substrate in trays and aerating it by forced draft; sensors control temperature, humidity, and the desired O_2/CO_2 balance.

Some solid-substrate fermentations are anaerobic. These fermentations, such as ensiling, do not need agitation or aeration. Agricultural products are fermented in tanks for 1-3 weeks at a temperature of 25°C. *Lactobactilis bulgaricus*

becomes the dominant organism. The production of lactic acid by this organism and anaerobiosis is unsuitable for other microorganisms.

Composting of animal and plant waste for mushroom production and other uses such as ensiling is carried out by indigenous microorganisms, but it depends on a succession of organisms to complete the breakdown of complex polymers such as cellulose and straw. Some of these organisms are not tolerant of high temperatures, so the substrate must be frequently turned over to keep the temperature low.

Solid-substrate fermentation employs a wide range or organisms, including numerous species of bacteria, fungi, and actinomycetes, mostly as pure cultures. Some of the more complex organic polymers are more effectively fermented by a mixed culture, as in composting. A two-stage conversion of straw using a mixed culture of *Chaetomium cellulyticum* and *Candida lipolytica* produces a larger fungal biomass than either fungus alone.

2. *Sterilization*.

If the product is to remain pure and uncontaminated with toxic substances, the fermenter, its ancillaries (including all pipework), and the medium must be sterile at start-up and maintained so throughout the period of fermentation. Dry-heat sterilization on an industrial scale is expensive and unsuitable for the media used; steam sterilization is used almost universally. Where components of the medium are particularly heat-sensitive, U.V. or radiation sterilization is used.

The fermenter and the medium may be sterilized separately or together. Separate sterilization of the fermenter has the advantage of allowing higher temperatures to be used for shorter times. This reduces the time during which expensive equipment is out of use. The fermenter is supplied with steam through a valve at the top of the tank, and air and water drained off at the bottom. When all the air and water are removed, the drain valve is closed and the pressure allowed to rise until the temperature reaches 120-160°C for the required time. The precise temperature used will depend on the sensors and seals used. As cooling begins, sterile air is admitted into the fermenter to control the drop in pressure within the tank and prevent its collapse. Cooling is effected by passing sterile cold water through the cooling coil or jacket. The use of sterile water avoids the risk of contaminating the sterilized fermenter, and the heated cooling water can be used to make up the medium or for some other part of the process to save energy.

The medium is sterilized in a separate closed vessel and is fitted with a coil for heating and cooling the medium, and with a stirrer that runs throughout the sterilization process to improve heat transfer throughout the medium. Steam is passed through the coil or jacket, and also is injected directly into the medium through the air line. When the vessel is purged of its air and the medium reaches 100°C, the exhaust valve is closed

and the pressure and temperature allowed to rise to bring about effective sterilization, but minimal damage, to the medium. Transfer of the sterile medium to an empty sterile fermenter is accomplished by raising the pressure in the vessel containing the medium with sterile air, or by simple gravity feed through sterile pipe lines.

In addition to the batch method of sterilization described above, a system for the continuous supply of sterile medium is used in some processes. This enables several batch fermenters used in sequence to be cleaned quickly, sterilized, and filled with sterilized medium, so that each one is only out of action for a short time.

Continuous sterilization methods also have the advantage of lowering the risk of damage to the medium, particularly in heat-sensitive mixes. The medium is first mixed, non-sterile, in a stirred vessel, such as the one shown in figure 18. It is then passed through a heat exchanger, where the temperature is rapidly raised to a suitable level (120°C-150°C), held at this temperature for the time required to sterilize it by controlling its rate of flow through the holding coil, and then passed on to a cooling coil and into the fermenter. Because the mass of the medium passing through the heat exchanger is small, it can be heated and cooled rapidly. This means the medium is exposed to high temperatures for a short time, resulting in less damage to the nutrients in the medium.

Joint sterilization of the medium and fermenter also follows much the same procedures as separate sterilization, except that sterilization of the medium is carried out in the fermenter and is therefore only suitable for batch processing. The medium is mixed up in a separate vessel, using the hot cooling water from a previous operation to save energy. It is then pumped into the fermenter, and the temperature is raised by injecting steam into the medium and through the cooling system. The temperature is held at 120°C-150°C for whatever parameters are found to sterilize the medium and the fermenter. The temperature is then reduced, taking the precautions mentioned above to avoid possible vessel contamination and collapse. It is very important to keep the stirrer running throughout the whole operation to assist heat transfer throughout the mass of the medium.

3. *Continuous Versus Batch Processing.*

The principle of the continuous process is to feed sterile medium in the fermenter at a constant rate into a stirred or air-lift tank. The products, medium, and microbes pass into a reservoir for extraction of the product and the cells. Alternatively, the fermentation mass may be passed into a second and third fermenter to undergo further fermentation, perhaps with different microorganisms. Where the cells are the required product, as in the production of single-cell protein for animal feed, the continuous fermentation process has had some success, but it has not been adopted widely on the industrial scale, where the advantages it seemed to offer in the laboratory have not been realized.

The continuous process has not been successful for two main reasons. First, the microorganisms are genetically unstable over a long period of culture, so the purity and yield of the product deteriorate. Second, some products, such as beers and lagers, owe their characteristic flavors to a mixture of yeast strains, and not to a single yeast. The proportions of the mixed cultures cannot be maintained over a period of time. Beer-making requires the separation of the anaerobic fermentation, which requires two or three continuous fermenters; this is complicated and expensive. The cylindrical tower fermenter, with aerobic conditions at the bottom of the cylinder and anaerobic at the top, could not compete commercially with the cylindroconical batch fermenter, which is faster and less expensive to operate and has the advantage over stirred fermenters, which require high energy input.

Enzyme Technology

Enzymes are too complicated to be synthesized commercially at present, so all the enzymes currently in use are synthesized by living organisms. They may be excreted by the cells that synthesize them into the environment (extracellular), or used within the cell for its own functions (intracellular).

Enzyme production was first centered on high-volume, low-cost enzymes for the food industries, and a little later on proteases for incorporation into household detergents. There is now, however, a gradually increasing demand for high-cost, purified enzymes for diagnostic purposes, immunoassays, and enzyme therapy.

Bulk, industrial enzymes are produced almost entirely by microorganisms. Although some 2000 enzymes have been identified, only about 20 are produced and used commercially, and currently only a small number of microorganisms is used in their production: 36 species of filamentous fungi, 5 species of yeast, and 12 species of bacteria.

The strain of the organism to be used for enzyme production is carefully screened for its ability to grow on cheap substrates, produce enzymes at a high and stable rate, produce no toxic substances, and have no antibiotic activity.

Extracellular enzymes make up the bulk of industrial enzyme production. The production of this form of enzyme has the advantage that downstream processing consists of a simple separation of the cells from the dilute enzyme solution. It avoids the difficult and expensive crushing of the cells to release intracellular enzymes.

Extracellular enzymes are predominantly produced in small (50m³), deep-tank fermenters of stainless-steel construction, fitted with mechanical stirrers and air spargers. The relatively small size of the fermenters is conducive to batch-culture techniques, which increases the ability of the fermenter to be used for the product of different products.

Production of the enzyme in the bioreactor is under some control. Apart from careful selection of the strain of organism used, the careful control of the physical parameters and a suitable medium, the innate regulatory mechanisms of the organism can be controlled to some extent by methods discussed in the section on the use of microbes in biotechnology.

1. *Immobilized Enzymes.*

Inexpensive, isolated, bulk enzymes such as amylases and proteases are added to the substrate and incubated at the optimum temperature, pH, and duration to produce maximum yield. Because they are water soluble, it is difficult to separate the enzymes from the substrate and the product, and the enzymes cannot be re-used.

By attaching enzymes to an inert support, they can be recovered by simple solid/liquid separation. This frees the product from the enzyme and makes the enzyme available for re-use in batch processes. Immobilization holds out the possibility of designed, multi-enzyme systems that can be used in continuously operated reactors.

Another possible advantage of enzyme immobilization is that it will give the enzyme more stability. Maintenance of enzyme stability is a problem in biotechnology. By covalently linking an enzyme to a solid support, the folded structure of the protein is less likely to be disturbed by heat, in pH changes, or other denaturing agents. Immobilized enzymes, therefore, should be more stable than when they are in their free state. Unfortunately, although immobilization provides stability in some cases, it does not do so as a general rule. Moreover, immobilization changes the physicochemical properties that exist in the free state; this impairs the function of the enzyme, particularly the diffusion of the substrate to the catalyst surface.

A. Methods of immobilization

There are three ways in which enzymes are immobilized.

1. Bonding. Enzymes may be covalently linked to an insoluble organic or inorganic support, as shown in figure 20. Covalent bonding has the advantage that it is not reversed by changes in pH, ion strength, or substrate. There are many methods for covalent bonding, and they employ many chemical groups such as amino, hydroxyl, carboxyl, thiol, imidazole, and phenol. The support substances are equally varied: porous glass and ceramics (amyloglucosidase), synthetic polymers (trypsin), cellulose (amylase), nylon (urease) and alumina (glucose oxiduse).

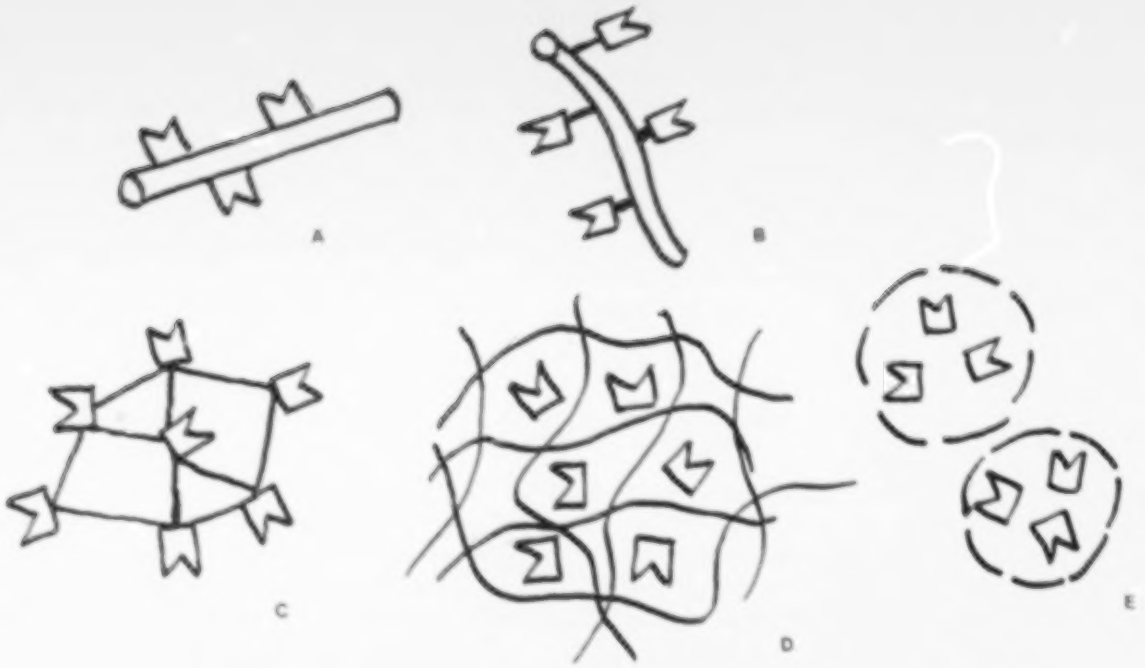

Figure 20. Immobilization of enzymes.
A. Physical absorption
B. Covalent bonding.
C. Cross linking.
D. Entrapment.
E. Encapsulation.

Covalently linking the enzyme to a support may cause some inactivation of the enzyme. Physical adsorption, however, is a simple process that does not involve reactive species of molecules, and there is, therefore, no distortion or chemical inactivation of the enzyme activity. Ion-exchange resins readily adsorb enzymes, but at the same time the binding is readily reversible. Other substances used are alumina (amylase), glass (urease), and hydroxyapatite (NAD pyrophosphorylase).

2. Cross-linking. Three-dimensional, water-insoluble aggregates are formed by cross-linking enzymes (figure 20). Cross-linking, however, is more usually accomplished on the surface of a carrier onto which the enzyme is absorbed (cellophane, for example).

3. Inclusion. This process includes two methods:

   a. Entrapment in a gel or filament network: the enzyme is added to a solution of a monomer, which is then polymerized, trapping the enzyme within the gel or filaments. Common substances include polyacrylamide, silica gel, silicone rubber, and starch.

   b. Encapsulation: Thin, semi-permeable membranes are used to enclose the enzyme. The membranes are permeable to low molecular weight substrates and products, but not to macromolecules. Materials used to make micro-capsules include nylon (trypsin and urease), polystyrene (catalase), and collodion (L- asparaginase). The enzymes are trapped in the cells by heat treatment or covalent cross-linking, and the cells are then pelleted.

Inclusion methods carry very little risk of inactivation of the enzyme. Encapsulation, however, does hinder free access of the substrate to the enzyme surface to some extent, and also impedes diffusion of the product.

The use of immobilized enzymes has not been very successful at the industrial level. The two most successful uses so far have been production of fructose syrup for use as a sweetener in the food industry, using glucose isomerase and glucose, and the use of penicillin acylase to provide 6-amino-penicillinic acid (6APA), which is used to synthesize several medically important antibiotics.

Techniques for Culturing Animal and Plant Cell

1. *Animal-Cell Systems.*

For some years animal cells have been cultured *in vitro*, using synthetic medium supplement with fetal calf serum to cultivate viruses for use in the preparation of vaccines. Several developments in the last twenty years have extended the use of animal cells for the industrial production of high-value products such as hormones, blood-clotting factors, interferon, and monoclonal antibodies. In the future, microbial cells (*E. coli* and *S. cerevisiae*) may be genetically programmed to produce these products. These microbes are easier and cheaper to culture in large numbers. At present, however, good progress is being made with animal cells, in spite of their more demanding culture requirements.

Both normal and transformed cells are used in cultures. Most normal cells require

a substrate to adhere to for successful cultivation; transformed cells do not require such a substrate and can be grown in suspension with little difficulty, using equipment similar to that used with microorganisms. Animal cells, however, require more carefully controlled environments.

Until recently, the presence of fetal calf serum was required in all animal-cell media; even today most cell lines are grown in media with a small concentration of serum to ensure optimal growth. The function of the serum is not completely understood, but it is thought to provide hormones, growth factors, low molecular weight nutrients, and trace metals. Serum also is thought to protect the cells against mechanical damage and to enhance the attachment of anchorage-dependent cells to surfaces.

The serum requirement creates problems for large-scale systems, because serum samples are not uniform in their ability to support cell growth. The age, sex, and nutritional state of the donor are some of the factors known to affect the quality of the serum. Moreover, the supply can be erratic, which makes steady, planned production difficult. Serum-free media have been designed for a few selected cell lines, and the consistent, controlled production of the media helps ensure reproducible results. The serum factors are replaced with expensive polypeptide growth factors and hormones, and antibiotics are added to maintain sterility.

Another critical factor in animal-tissue culture is the purity of the water used to produce the medium. Cell-growth inhibitors are widely present in water supplies, and they must be removed totally; usually this is accomplished by reverse osmosis.

Animal cells have other restrictive requirements: they tolerate only a narrow range of pH (7.2-7.4), so the medium must be well buffered, and they must be well supplied with oxygen and an energy source, usually sucrose or glucose, depending upon which growth phase yields the greatest amount of product.

A. Cell-cultivation systems

Two types of cell cultivation are used in large scale systems: mono-layer and suspension cultures.

1. Monolayer culture--used for anchorage-dependent cells--requires a surface to which cells can adhere, and because normal cells attached to a surface will not grow over each other, the number of cells in a cultivator depends upon the surface area available to the cells. Once the surface is covered completely, cell growth ceases. This feature of normal animal cells has created problems for large-scale systems.

A number of methods have been tried to produce a large surface area in a small volume (figure 21). These include roller bottles, stacker plates, spiral plates, and artificial capillaries. All of these systems must be perfused with fresh medium, and the pH and CO^2 levels monitored carefully and maintained within close limits.

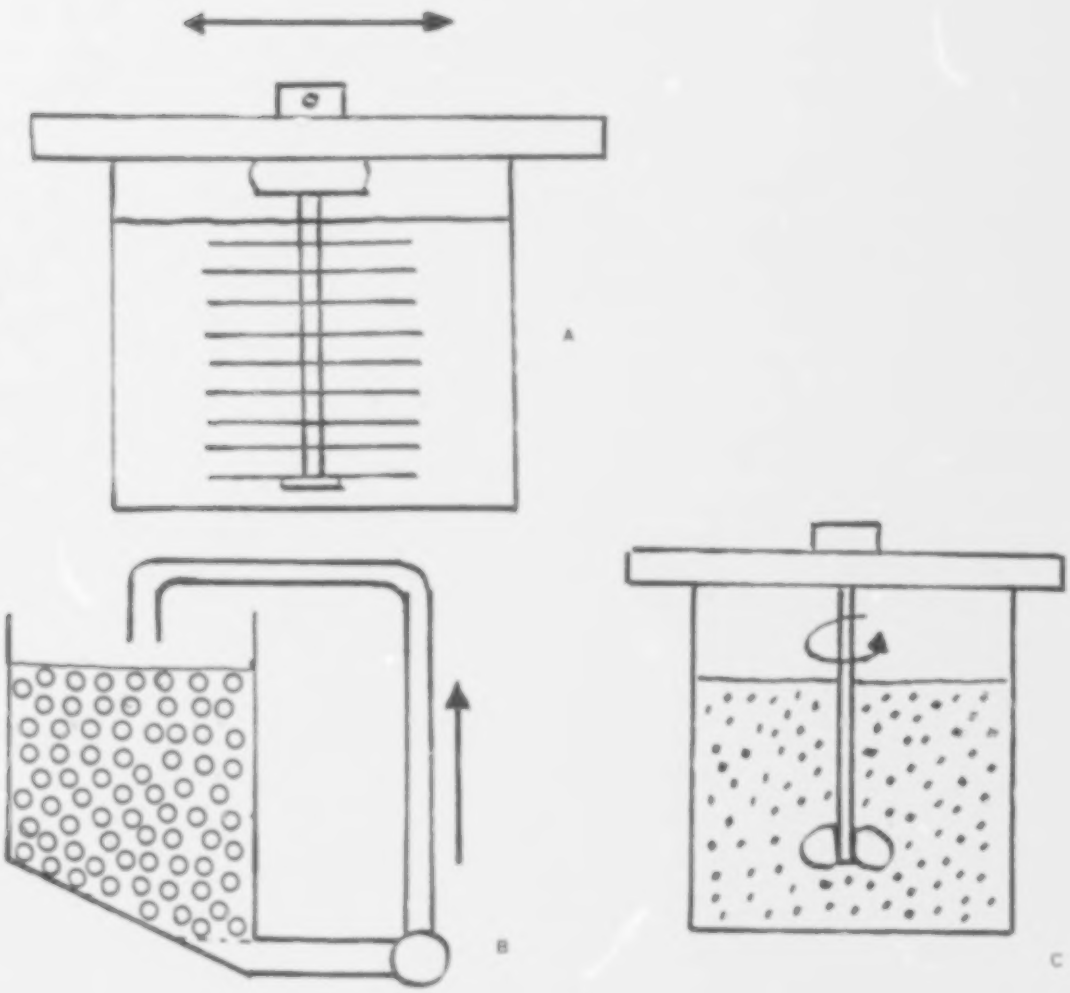

Figure 21. Methods to increase surface area for mono-layer culture.
A. Oscillating plates. B. Beads. C. Micro-carriers.

More recently, the productivity and ease of handling of such cultures have been improved by using micro-carriers in the form of small beads that can be held in suspension in the liquid medium by gentle agitation. These can be used in densities of 5-25,000/cm, providing a very large adhesion surface. A wide range of materials has already been used to make the beads, including polystyrene, polyacrylamide, and crossed-linked gelation; all beads are coated with fetal calf serum. The most widely used micro-carriers are cross-linked dextran beads, which can be used in concentrations per liter that provide a surface-area-to-volume ratio of 120-150. Fairly standard bioreactor systems can be used for cell cultures supported on micro-carriers, but low stirrer speeds are necessary to avoid shear effects and cell detachment. Air is not usually bubbled up through the suspension, because the medium is prone to foaming. Instead, pure oxygen is added to the surface of the culture, and reaches the cells by diffusion through the stirred medium. These suspension systems can be used in bioreactors of several thousand liters, as in the production of interferon from human fibroblasts. The system allows easy medium changes and washing of the cells.

Non-anchorage dependent cells can be cultivated in suspension culture systems similar to those used for microorganisms. Large-scale systems have been used for many years to produce vaccine for foot-and-mouth disease from hamster kidney cells suspended in 3,000-liter stirred-tank reactors. Transformed human cells, which behave like cancer cells in that they are not anchorage dependent, have been used for the production of high-grade pharmaceutical products. When transformed cells are used, the highest standards of product purification and testing must be enforced to exclude the possibility of the transfer of some harmful transmissible agents with the product.

2. *Plant-Cell Systems.*

The first plant cells to be cultured *in vitro*--about seventy years ago--were from meristem tissue excised from the roots of grass seedlings and grown in a liquid-culture medium of inorganic salts and glucose. The root tips grew vigorously at first, but they could not be maintained, even when subcultured. Asceptic techniques and improvements in the medium--first by the addition of B-group vitamins, and later by the discovery of the beneficial use of coconut milk--led to the sustained and successful culturing of shoot and root tips, leaves, and embryos. The beneficial effect of the coconut milk was soon traced to the presence of the plant-growth substances--auxin and cytokinin. By changing the proportions of these two substances in the medium, the differentiation of undifferentiated masses of callus tissue in culture could be directed into the formation of shoots, roots, or both.

The objective of much of this work was the establishment of cultures of separated cells. This was first achieved by shaking fragments of callus tissue in a liquid medium, and later by the mechanical disruption of leaf mesophyll. Further opportunities for exploiting plant tissue culture were created by the enzymatic solution of the cellulose cell walls of plant tissues to form single cells, and by the release of protoplasts from single cells. Diploid and haploid plantlets were soon produced regularly from the single cells of vegetative tissue and pollen cells. The haploid protoplasts offer the prospect of hybridizing sexually incompatible species; of introducing beneficial microorganisms into plant cells by fusing the protoplasts of the two organisms; of easing the problem of manipulating the more complex plant genome to produce plants beneficial to agriculture; and of using plant cells in suspension cultures to produce high-value products such as steroids, codeine, digitalin, and anti-tumour alkaloids.

Plant tissues now are being used in three ways: the *in vitro* propagation of plants; the production of secondary metabolites from mass cell cultures; and the use of recombinant DNA techniques to modify plants genetically.

A. *In vitro* propagation

This technique is well established in horticulture and forestry. After surface sterilization, very small (0.1 mm) pieces of the apical meristem are placed on a semi-solid substrate of agar made up in a suitable sterile nutrient medium in a sterile flask. The meristem tissue multiplies rapidly, and in the presence of excess cytokinin relative to auxin, the meristem undergoes extensive branching. The clusters of shoots can be sub-divided and recultured many times. At intervals, some of the shoots are transferred to a rooting medium (more auxin relative to cytokinin) to produce roots. When the roots have developed, the small plantlets are transferred to a growing compost and hardened off. This procedure can produce a large number of plants and forest trees rapidly. Pieces of callus tissue, which can be produced in culture from very many species of plants, can be treated in the same way. The callus tissue multiplies so fast that even virus-infected explants soon produce virus-free cells.

These methods have been very successful in producing large numbers of disease-free stock in a few months, instead of the years it takes with more usual vegetative and sexual means in many forest and orchard trees. Micropropagation allows plants to be produced unrestricted by climate or season. The rapid production from a few plants of a new variety produced by conventional crossings, and the prospect of mass production of new varieties are derived from recombinant DNA techniques.

B. Production of secondary metabolites

Techniques for maintaining plant cells derived from plant organs (leaves, shoots or roots) or callus tissue in suspension are well established, and there are well-defined media that consist of mineral salts, an energy source, vitamins, and plant-growth regulators. These media are designed to suit the type of cell being cultured, and the products required are generally less complex, cheaper, and easier to handle than media required for microorganisms or animal cells.

The methodology used is similar to that used for microbes. The cells are cultured in tank-fermenter vessels, but their large size, fragile nature, and tendency to aggregate does cause some problems. Air-lift bioreactors are more commonly used than flat-bladed stirrers, as the former do less damage to the cells.

Plant cells are totipotent, and any cell, therefore, should be capable of producing the typical product of the species. Experience shows, however, that it advantageous to use only high-yielding, disease-free strains to initiate the cultures.

Plant cells grow more slowly than bacteria. Doubling times are about 20-40 hours, as compared with 30 minutes for bacteria. High growth rates, however, are often not desirable, as many of the useful plant products are secondary metabolites that are only synthesized by resting or differentiated cells. The effects of medium constituents are now under investigation. Glucose often provides the fastest growth rate and maximum biomass accumulation, but it may inhibit product yield. For this reason, sucrose, which is metabolized more slowly, is used as an energy source in these situations. Similarly, the form and the proportions of nitrogen and phosphate sources, along with the concentration of growth factors, have important effects on product yield. Suspension cultures in small-scale production systems have been found in many cases to give similar or even greater yields of products, when compared with the parent plant. So far, the use of suspension cell cultures for the production of plant products has not been readily adopted commercially, yet some of these products (vinblastine, digitalis, insecticides, perfumes) are of high value and have sizeable markets. The first and only commercially successful product to date is shikonin, a dye and pharmaceutical agent.

Because most secondary plant products are under control of multiple genes, the genes for such compounds are unlikely to be cloned into microorganisms in the foreseeable future. Work is therefore concentrated on selection strategies for identifying high-yielding cell lines, on the removal or reduction of catabolite repression, and on the use of strong promoters.

C. Recombinant DNA techniques

Gene cloning is very much in its infancy in plants, but the search is on for suitable vectors such as the plasmid and phage vectors of bacteria. Plant viruses are being examined for their suitability as vectors, and some promising results already have been obtained using the Ti-plasmid from the bacterium *Agrobacterium tumefaciens*, which causes the development of the crown-gall tumor in many dicotyledonous plants.

The Ti-plasmid introduces a segment of DNA (T-DNA) into the plant chromosome, and there codes for a range of amino acid derivatives called opines, which are not normally found in plants. These opines can be used by Ti-plasmid-bearing strains of *A. tumefaciens* as a source of carbon, nitrogen, and energy, thus enabling the bacterium to multiply in the plant. The T-DNA also alters the ratio of auxin to cytokinin, leading to the growth of the plant tumour. This T-DNA is able to maintain the tumour in tissue culture without supplementary growth substances or continued infection by the bacterium. In other words, the bacterium performs a true, natural genetic manipulation of the plants. It is hoped that this system can be used to introduce new genes into plants.

So far, octopine (one of the opines)-synthesizing activity has been introduced into plants that are normal and healthy, and with no detectable T-DNA. In another set of experiments, a nopaline plasmid has been used successfully to introduce a yeast alcohol dehydrogenase gene into healthy tobacco plants, but the gene was not expressed. An active galactosidase was obtained, however, in sunflower and tobacco plants, so the system does seem to hold the prospect of introducing useful genes into plants.

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Additional Reading

1. Biotechnology in Focus
   Katz, J. and Saltelle, D.B. 47 pp.
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2. Biotechnology in Perspective
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Chapter 3

SOCIAL IMPLICATIONS OF BIOTECHNOLOGY

Introduction

It is now widely recognized that science education is no longer solely about learning science--its concepts and the methods used by scientists. The social implications, both personal and collective, of scientific ideas and their technological applications are perceived as equally important and are becoming more prominent in science curricula at all levels. The advent of biotechnology has contributed to and intensified this trend.

The social implications of biotechnology are mirrored, for example, in concerns about the manipulation of the human genome and the release of genetically engineered microorganisms into the natural environment; indeed the term genetic engineering itself frequently stirs strong, emotional, negative opinions (Research and Forecasts, Inc., 1987). On the other hand, the obverse side of such issues--in the case cited, the prospect of eradicating genetic ailments and producing more and cheaper food--can stimulate equally strong supportive attitudes. Such is the impact of biotechnology that it has galvanized governments, industrialists, scientists, environmentalists, religious leaders and many others to spawn conferences, committees, reports, and, occasionally, policies. It has initiated a debate that will clearly continue into the adulthood of our students. They need to learn about its content and develop the skills and attitudes that will enable them, in their turn, to contribute to the debate and make the decisions and take the actions that arise from it.

Educational Goals

The social implications of biotechnology have much in common with those of other topics usually included in school science education, for example, environment, behavior, and reproduction. Teaching about such topics shares the following, general educational goals:

1. To ensure that students are aware of the close relationships between science, technology, and society in its many manifestations--economic, cultural, moral, legal, and political.

2. To stimulate students to discuss--and continue discussing--issues concerned with science, technology, and social policy. In particular, to encourage their tolerance of diversity of opinion and ambiguities in decision making; to think out issues, not just to react to them.

3. To encourage students to evaluate their own values--the moral frameworks they may tend to take for granted--in relation to developments in science and technology.

Requisite Understanding and Techniques

To fulfill the general goals listed above, we must introduce students to seven major areas of understanding and technique.

1. Sound Knowledge of the Science Involved.

One cannot analyze, discuss, or decide without adequate knowledge. Poor understanding of science will make for poor analysis of social issues. Unless one knows the facts, and the validity and relevance of the facts of an issue, judgment will inevitably be inadequate. To deal with the issues of biotechnology, students must first learn about the science involved and then, subsequently, seek and incorporate further knowledge as it is needed. The subject matter outlined in Chapters 1 and 2 is fundamental to dealing with the societal implications of biotechnology.

2. The Differences and Relationships of Science, Technology, and Social Issues.

Notions such as "technology is applied science" or "social problems can be solved by scientific methods" can be misleading in that they imply there are no differences between science, technology, and mechanisms for analysis of social problems. Science, however, is a process of rational inquiry that "seeks to propose *explanations* for observations of natural phenomena," while technology "proposes *solutions* to problems of human adaptation to the environment" (National Center for Improving Science Education, 1989). Both science and technology are concerned with objective phenomena related to the material world, while social problem solving is inevitably subjective in its outcome, relating to emotion and opinion, as well as fact. As the biologist John A. Moore (1984) has written, "Science can tell us what we *can* or *cannot* do, but is helpless to tell us what we *should* do."

Of course, scientists, technologists, and the rest of society are humans. Their different professions do not make them totally different people. In this respect, we can expect similarities in the way they tackle problems. Nevertheless, by emphasizing the differences between science, technology, and social problem solving, students will come to understand the relationships between them. Such distinctions improve clarity of thought and are particularly important when dealing with the social implications of biotechnology, which invariably incorporate aspects of all three.

One can see the distinctions by taking the case of antibiotics, such as penicillin, which act therapeutically by controlling disease, or when mixed into cattle feed result in increased growth of the animals. Identification of the structure and function of antibiotics involves the procedures of science, essentially experimentation. On the other hand, the mass production of an antibiotic for a particular purpose involves engineering and design principles, and essentially is technology. When we come to consider that most of the antibiotics used in animal feeds also are used in humans, to help combat diseases, and that their use in feeds could build up antibiotic-resistant strains dangerous to humans, we have to decide what to do next. Then we are dealing with social implications. The three aspects are clearly linked, but the issues that arise from them are different, certainly in their objectives, and to a considerable extent, in the procedures involved.

3. Procedures of Problem Solving.

Education that is concerned with the social implications of biotechnology inevitably is concerned with problem solving. At the same time, it will be set within a context of science and technology from which the students will derive an understanding of the objectives and procedures characteristic of those disciplines. We already have suggested that students must be aware of the *differences* between the three activities; on the other hand, not to be aware also of the inter-relations and *similarities* can lead to confusion, because any consideration of social implications will invariably require reference to scientific and technological issues as well. Table 1 summarizes the similarities and differences between science, technology, and problem solving, all three of which involve six types of procedures. The ways in which the procedures are undertaken vary, but there are similarities in the intellectual and other skills that are used.

On cursory examination, it appears logical that the six procedures follow each other in sequence. In fact, it is useful to portray the sequence in that order for students as a guide to study. In reality, however--and this will soon be revealed to the students in their work--there is overlap. Evaluation, decision making, and data analysis, for example, contribute to the definition of goals as one refers to related topics from which to gain ideas. Process operation and data analysis (at least in part) often occur simultaneously, or nearly so.

The fact of overlap highlights the importance of providing students with the experience of undertaking activities that involve all the procedures and not just exercises related to individual procedures. This approach also increases student motivation. Students should feel that they "own" the problem they are dealing with and that they are dealing with concrete realities or, at least, realistic simulations of them.

Problem-solving activities are best undertaken as cooperative group work, enabling students to develop personal skills such as those of *communication* (listening as well as explaining), *empathy* (understanding another person's point of view), *organizing* (ensuring

that a maximum number of considerations are taken into account), and *controlling judgment* (waiting until adequate facts and opinions have been considered; accepting the possibility of variety or uncertainty of conclusions).

Procedure	Science*	Technology	Social Implications
Goal definition	Devising hypotheses	Specifying product	Defining issue
Process design	Designing experiment	Designing productive process	Determining problem-solving strategy
Process operation	Conducting experiment	Producing product	Solving problems
Data analysis	Assembling quantitative and qualitative data in a form that can be used for evaluation; distinguishing facts from values and opinions		
Evaluation	Appraising data in relation to goals, limitations of procedures, and relevant values; creating and using a variety of possibilities for interpretation and inference		
Decision making	Devising decision-making trees; making judgments; relating to other factors and judgments; translating to action		

Table 1. Similarities and differences between the methods of science, technology, and analysis of social implications.

*For the sake of simplicity, science is interpreted as experimental science.

Underlying all aspects of thinking about the social implications of biotechnology is the need for students to be aware of the nature of judgments and the way we arrive at them. Judgments are made in all aspects of problem solving encountered in studying biotechnology--in activities as varied as reading a graph, choosing between hypotheses, designing an industrial process, or making decisions about social action. It is important that we make valid judgments and realize their limitations. In general terms, this requires us to heighten the students' awareness of the way they think and what, from their memory, experiences, and emotions, they and others concerned with making a judgment bring to bear on that judgment. This is not an attempt to turn students into philosophers and

psychologists, although they should realize that such disciplines have a role to play in dealing with the social implications of biotechnology. It is, rather, to show students that the quality of a judgment rests firmly on the quality of the contributions and contributors to it. To demonstrate this point, the following questions should be brought to the students' attention as they discuss issues or work out problems.

Question 1: Is the type of thinking employed systematic and constructive, or open and unstructured? The former would involve, for example, the logical development of each step in an argument, the deliberate playing with logic to produce ideas (such as with lateral thinking, when one deliberately tries to look for unconventional explanations), and the establishment of concepts against valid criteria. Open and unstructured thinking does not imply laziness; that clearly has little value. If, however, there is a strong level of commitment, open thinking can be creative, relying to a considerable extent on intuition and the production of ideas without conscious reference to their origins. Such thinking can result in inspired and original thought. Without some association with the discipline of systematic thought, however, it is unlikely to be effective.

Question 2: Are the students conscious of the validity and limitations of the sources and relationships of ideas? An example of the issues raised by this question is the distinction between knowledge (data) determined by direct experience such as *observation*, and that which is second-hand, produced by relating different elements of observed data or assuming the derivation of one concept from another or from an observation, that is, *inference*. That some people have hemophilia is clearly an observation. That, when one traces its distribution in families over several generations, hemophilia follows a Mendelian pattern of inheritance also is an observation. The conclusion that hemophilia is genetic in origin is, however, an inference that rests on the assumption that a Mendelian pattern of inheritance is proof of genetic origin. In fact, by itself, a Mendelian pattern merely indicates the possibility of genetic origin; it is not direct evidence, as is an observation. In this case, consistency of findings of many studies indicates it is a valid inference and makes a sound working hypothesis for further study and action. In other cases such as that of Alzheimer disease, which in fact affects more people, the inference is limited, because the distribution of the disease does not suggest such a clear genetic component. That it is genetically determined is only a possible hypothesis.

Observation and inference produce different types of knowledge. We tend generally to have more confidence in observation, because it produces knowledge directly through our senses or extensions of our senses, as when we use instruments such as microscopes. Nevertheless, observation is subject to error and limitations. Observation can be controlled by perception. We can be unconsciously selective in what we observe, as any student looking for the first time at a slide of a cell will acknowledge. Our senses also have a limited range; indeed, that is why we use instruments such as microscopes. Furthermore, instruments also are subject to technical error and limitations.

One can judge the value of an observation or inference by asking whether it helps to explain other phenomena. The validity of this question can be seen by considering the particular form of inference depicted in the use of a model. For example, the spiral DNA model of Crick and Watson represents what was inferred from chemical tests, crystallography, and other procedures. The genius of this inference was to represent the data in a form--the model--that provided the means to explain chromosomal division, replication, and other phenomena (Watson and Crick, 1953; Crick, 1988). The model also provided the basic ideas on which further research could be based, and that has led, in fact, to genetic engineering and modern biotechnology.

A comparable distinction to that between observation and inference is between *causation* and *correlation*. We can, for example, show that people who have certain characteristics, for example, Down syndrome, tend to have abnormal chromosomes in their cells. The association is an example of a correlation: when one factor occurs, so usually does the other. It is a big jump, however, to infer that this represents causation--that the abnormal chromosomes cause the characteristics. The developmental processes linking chromosomes to the structure and behavior of a whole person are exceedingly complex. It may well be, for example, that environmental factors contribute to the development of the characteristics in question. In a sense, this has to be the case, because the substance of a person clearly does not come only from the chromosomes in a fertilized egg. It is possible that one day we will be able to trace the complex series of causal events that relate chromosomal aberrations to a person's characteristics. Until then, the relationship remains a correlation.

Awareness of distinctions such as those between observation and inference or causation and correlation encourages clarity of thought in students and ensures that they check on the validity and limitations of their thinking.

Question 3: Are students aware of the influence of the time frame of an issue? One field for which biotechnology has particular implications is genetic counseling. This is the procedure by which potential parents are advised of implications for their children arising from their genetic makeup. Until recently genetic counseling relied almost exclusively on deductions based on Mendelian concepts of inheritance, which provide estimates of mathematical probability for the presence of characteristics in offspring. On the basis of those estimates parents would have to decide whether to conceive children, and, in cases where pregnancy was in progress, whether to carry the pregnancy to term or choose a therapeutic abortion. Developments in biotechnology now permit detection of many adverse characteristics at early stages of embryonic development (Childs, et al., 1989). This offers the opportunity for more accurate decision making, but inevitably raises the issue of the morality of abortion--the removal and consequent death of the fetus. If one defers a decision until after conception, genetic counseling must raise the issue of abortion.

As time has passed, scientific understanding has grown and changed, and as it has, the form and context of social (and personal) issues arising from it also have changed. This example, as will many others, shows by extension that our judgments today will not necessarily be based on the same understanding or, indeed, be of the same kind, as those we make in times to come. The need to revise one's judgments on the basis of new data or technology does not imply that previous decisions were somehow incorrect or ethically deficient. Rather, it reinforces the notion that one must make judgments on the basis of the best information available at the time. Students should be fully aware of this time perspective.

Question 4: Are students aware of the influence of their emotions and values on their thinking? There is little doubt that reference to the case of genetic counseling just outlined will stimulate discussion between students. How much, however, will be focused clearly on scientific aspects and how much will reflect their preconceptions, thei. values derived from religious or other view points, and their emotional reactions to concepts such as abortion? It is not always possible or valid to confine discussion of social implications to scientific matters, but we should at least be aware of the extent to which we make judgments related to value-related and emotional criteria as opposed to scientific ones. It is critical, therefore, that students be able to distinguish facts from values and opinions.

4. Risk Assessment.

Risk assessment--the determination of the likelihood that something unfavorable will occur as the results of an action or event--is a form of problem solving that has particular relevance to societal issues related to science. This was an issue raised early in the development of rDNA technology, which used the bacterium *E. coli* because so much was known about its genetic structure and function. Because *E. coli* occurs naturally in the human intestine, some people--scientists and nonscientists alike--were concerned that new, technologically produced forms of *E. coli* might escape, infect humans, and have harmful effects (Davis, 1986).

The issue was tackled by conducting a number of experiments that involved feeding recombinant organisms to laboratory animals and noting the effects. The risk of harmful effects was determined in the form of probabilities--the number of cases of harm compared to the total number of animals affected. On the basis of previous experience, the scientists involved assumed that the animal experiments would mirror effects likely in humans. Assessments were made of the risk involved, and consequently some procedures were approved, others abandoned or modified. *E. coli* itself was modified to ensure that it would not be viable outside the laboratory. In addition, biologists developed standard procedures for containment of microorganisms based on the potential danger of the experiment or procedure in question.

Risk assessment involves *quantitative* factors--usually in the form of statistical probabilities--but also *qualitative* factors. This latter is the case, for example, when the precautions needed to overcome a hazard are such that they affect the quality of life of those involved, as might be the case if those exposed were required to follow elaborate hygienic procedures or to vacate their homes. Such qualitative factors can be *perceived* risks, that is, what *might* be the case as much as what it actually is. The validity of perceived risks should be scrutinized carefully.

Risk assessment consists of three aspects: risk analysis, risk comparison, and risk/benefit analysis.

A. Risk analysis involves procedures such as those outlined in the case of *E. coli*. The procedures consist of identifying the kind of risks involved--for example, scientific, personal, social, economic, legal, and safety--and assessing them quantitatively and qualitatively as appropriate. Note that the term "risk" applies to different forms of potential harm--economic, cultural, and legal, for example--as well as to safety and matters of physical harm.

B. Risk comparison is needed when, for example, alternative methods are possible for a particular biotechnological procedure. The risks applicable to each method must be compared. A different form of risk comparison is required when analysis is so complex that a single, simple answer is not available. In such cases alternative scenarios are composed and compared; worst-case and least-case scenarios are examples.

C. Risk/benefit analysis sets the risks involved against the benefits that may accrue from the event or action. The benefits may be economic, social, or cultural, as well as scientific. In the case of a biotechnology industry based on rDNA procedures, the benefits could include the medical benefits of drugs produced, and the increased employment produced by the industry, which might stimulate the economy of the local community.

Risk management follows risk assessment and involves determination of a policy based on the evidence and arguments arising from it. Risk management involves consideration of the following questions:

a. Who should determine policy and carry it out?
b. What evidence is available? Is it sufficient?
c. How valid are the assessment and analyses used? What are their limitations?
d. Are the methods used to resolve value-related judgments valid?
e. What is the balance between benefits and the risk adopted?
f. What is the relationship between assessments, value-related judgments, and the proposed actions?

g. What are possible future implications of the proposed actions?

We will examine such matters more fully later in this chapter, under the topic of policy making.

5. Futures Study.

As with much of science, technology, and society, issues to do with biotechnology invariably relate to the future. "Future" is the unspoken but implicit word in many a statement about the subject: "The impact of biotechnology on human welfare will lead to the eradication of cancer" (in the future); "Mankind treads dangerous ground in embarking on genetic engineering" (in terms of the future). These are examples of much that is said. They all relate to the future, and the contentions they state can only have validity in the future. Indeed, it is only in the future that we will know whether they have any meaning at all. There is, then, a need for students to develop a rational and exploratory perspective about the future in their studies. The coordinated, international effort to sequence the human genome, for example, is expected to be completed shortly after the turn of the century, when today's secondary school students are young adults. They, then, will feel the effects--positive and negative--of the detailed illumination of human biological variation at the molecular level.

Risk-benefit analysis, which has previously been outlined, is a future-oriented procedure. It asks, "What will happen if...?"

More generally, there are three alternative ways to explore the future:

a. Forecasting, by which processes and events identified in the past and present are extrapolated into the future;

b. Developing, by which, through careful estimation, scenarios are created that portray possible events and situations in the future founded on credible developments from the present.

Neither forecasting nor developing claims certainty because the future is still to be determined; the elements in that determination are not necessarily apparent.

c. Prediction, which claims certainty; it should be used with the greatest of care.

Several methods used for forecasting and developing would be valuable for student studying biotechnology.

Methods Used in Forecasting

Extrapolation. This method relies on records of trends over recent years, usually in the form of graphs, and then extends them, following the past patterns, into the future. Examples of topics for which extrapolation would be directly applicable include employment rates and production rates in the biotechnology industry, and rates of success with medical treatments. The possible impact of new biotechnologies on current, related trends also can be studied through extrapolation. The difference between agricultural production of a specific crop before and after the introduction of biotechnological methods is a case in point.

Precursor analysis. Developments in biotechnology depend, for example, on research findings, technical developments in instrumentation, financial support, and public and political approval. By examining aspects of scientific research today, it is therefore possible to discern ideas and methods that are likely to foster future developments in biotechnology. It was, for example, a reasonable speculation following the elucidation of the structure of DNA that genetic engineering would eventually be a reality. Knowledge of the structure of DNA was a precursor of genetic engineering. Identification of current precursors--for example, the polymerase chain reaction, the allotment of funds for the biotechnological industry, or government approval of new developments--can validly point to future possibilities. Of course, the same is true, although in a different direction, if one or more precursors is absent or contrary to development. The absence of tailor-made restriction enzymes is a relevant example, as is the absence of informative DNA probes for certain genetic disorders.

Methods Used in Developing

The reference to precursors indicates that future developments in biotechnology depend on a variety of factors. It is not a simple, linear process, but one that involves the combination and interaction of factors derived from science and from many other spheres of influence. Methods aimed at developing future scenarios need to take this into account, and a relatively simple method that students can use for such analyses is a cross-impact matrix, as shown in figure 1. This enables one to examine the possible interactions between two lines of possible future development such as, for example, technical developments in biotechnology and the economic status of a given country. These two lines form the axes of a matrix.

As an example, we can take the case of possible developments in the American human genome project, the attempt to sequence the genes on the human chromosomes. This project has now been established, and the rate of progress will depend on the extent technical developments occur and on the funding provided for the project. The latter will,

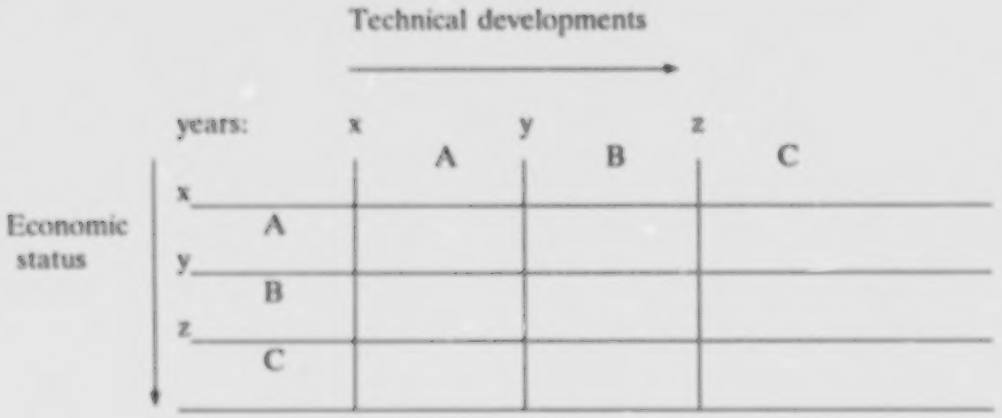

Figure 1. A cross-impact matrix.

of course, depend on the number of alternative requirements that make demands on the funding available.

A possible sequence for technical development is:

a. the development of automated techniques for analyzing the structure of the chromosomes; this should reduce costs considerably;

b. the development of a computerized data base; this will add to costs;

c. the mass production of independent genes and gene sequences capable of being freely transferred between chromosomes of humans and other organisms; this could be of value to the pharmaceutical and other industries.

In the light of current appraisals, it is likely that these three developments will occur in the sequence A--B--C, although with some overlap.

Over the same period of time it is possible that public funding will be less available for the project because of demands for research in other fields, especially the environment. Industrial funding will be available when the project produces commodities that can be used commercially.

These two trends can be depicted along the axes of a cross-impact matrix, and possible results of the interaction between the two trends can be depicted in the internal cells. A simple example of this is shown in figure 2.

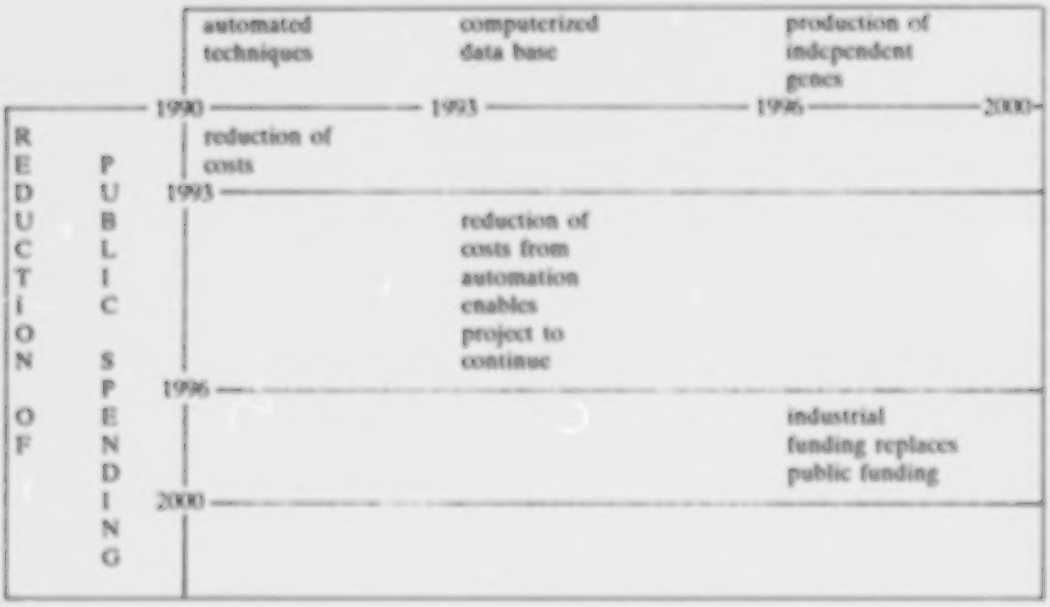

Figure 2. Cross-impact matrix of possible developments in the human genome project.

More detailed and sophisticated analyses can be undertaken and alternative matrices compared. As students complete a matrix they should be apprised of the limitations as well as the validity of their judgments.

The value of the cross-impact matrix is that it imparts a level of rigorous analysis into depicting the future and presents a number of short-hand descriptions of possible futures. Scenario writing is a less rigorous method, but one that provides more opportunity for creative judgment. One can combine this method with a cross-impact matrix, providing much fuller descriptions, synthesizing the products of the cells collectively, and identifying and commenting on inconsistencies. Scenario writing provides for a much fuller use of language. Alternative scenarios can be constructed when, as is often the case, there is no single, exclusive conclusion to be drawn.

Compilation of possible future scenarios related to developments in the field of molecular electronics would be of value to students and, at the same time, would introduce them to some of the methods of futures study. Figure 3 outlines the history and points to the future of molecular electronics--the science that attempts to link biological and electronic systems. It has its roots in both biotechnology and microelectronics.

Molecular electronics is responsible for the development of organic biosensors in which, for example, a monoclonal antibody is used to recognize and bind to a specific protein. Recognition involves the production of an electrical charge, which, in turn, causes

a current to flow in silicon and hence is incorporated into the circuitry of a computer. Other forms of biosensors are used in artificial sense organs and as control devices for heart pace-makers.

Research on devices known as field effect transistors has produced transistors that respond to the electrical potential of ions and hence are analogous to neurons. This has

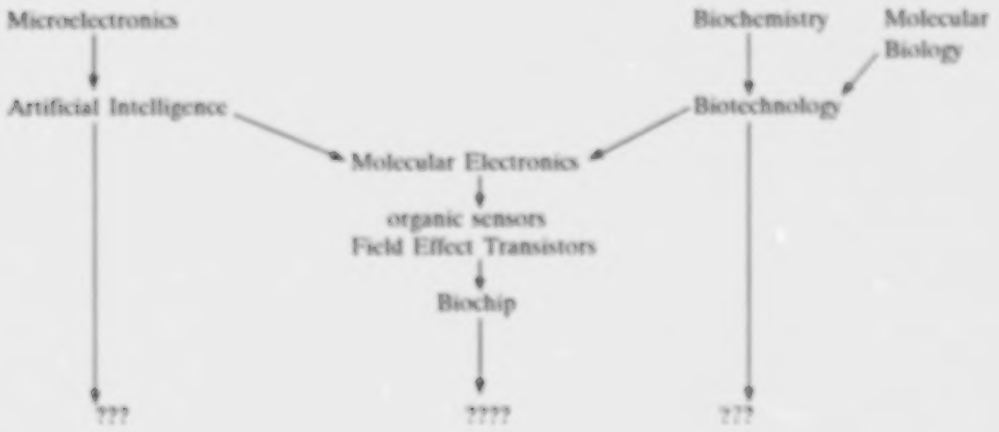

Figure 3. The history and future of molecular electronics.

led to research on the "biochip," the use of organic molecules instead of silicon chips in computers. Such research also has led to speculation that genetic engineering can produce computer circuits and to further speculation on what will result when biological and electronic systems can be integrated more fully. Will longevity or even a form of human immortality become a possibility when the functions of the body can be taken over by new biotechnologically produced parts or are replaced by bioelectronic artificial parts? Will we have to redefine our concept of "natural?" What are the demographic implications of increased longevity?

By tracing progress in research from scientific papers or even popular science journals, students can elucidate the precursors of possible future developments. A cross-impact matrix can relate lines of development in biotechnology to developments in electronics or, similarly, possible lines of development in molecular electronics could be matched to possible developments in social attitudes. Students could be encouraged to compare possible future scenarios based on these analyses and speculations. They also might refer to relevant science-fiction literature.

Such studies would be valuable in pointing students' minds to the future; they are, in fact, the type of studies used to help formulate policy. This also is relevant. If the studies are undertaken with care and a reasonable acknowledgement of their limitations, the students will gain great benefit from the anticipation of their possible futures.

6. Ethics

Although not explicit in the foregoing discussion, the role of morality (distinguishing good from bad, and right from wrong conduct) in dealing with the social implications of biotechnology has been implicit in the numerous references to values. A brief consideration of the human genome project provides another such case.

When the project was first contemplated, two types of questions arose: "Is it possible?" and "Ought it to be undertaken?" The first question is essentially scientific; it asks whether our understanding of genetics and molecular biology, and our technological capabilities, are sufficient for the task. The second question is about morality; it asks, for instance, whether we have the right to interfere with such a profound element of the human organism. Now that the first question has been answered in the affirmative and the project has commenced, the debate revolves around the consequences that may arise when the details of the genome are known (National Research Council, 1988; OTA, 1988). This, potentially, would make it possible to find a cure for cancer and to eliminate single-gene disorders. It might also provide opportunities to alter the characteristics of humans in unacceptable ways. A person need no longer develop as a result of the chance process of sexual reproduction, but could be produced by deliberate design. Such possibilities raise many moral issues. In many respects, morals have as much relevance to biotechnology as does science. For future biologists and citizens alike, the moral implications of biotechnological developments are of paramount significance and, therefore, it is important that our students understand something about the nature of morals and the related nature of ethics.

In ordinary language, the terms moral and ethical are frequently used synonymously. Here, however, we will distinguish between *morality*, as the phenomenon of right and good or wrong and bad as it applies to people and their actions, and *ethics* as the process by which we analyze moral issues, come to decisions about them, and take consequent action. To deal with moral issues, a person must understand and have the ability to practice ethics.

The introduction of an ethics component either within or associated with a biotechnology course cannot be taken lightly. Discussion of moral issues can easily become a mere rehearsal of preconceptions and prejudices without much thought and of little consequence. Students usually find moral issues interesting, but they must appreciate that such issues are as difficult to deal with as scientific problems. Ethical analysis, like scientific inquiry, requires rigor of thought, although partly of a different kind from that applied to scientific problems.

As a framework for dealing with moral issues, students should be aware of three particular aspects of ethics: that people have different attitudes towards ethics; that there are different presumed moral codes that can circumscribe ethics; and that there are recognized strategies for ethical analysis.

A. Attitudes depict a person's general disposition towards the notion of ethics itself and the systems of dealing with moral issues. At least seven types of attitudes can be discerned (Beauchamp and Childress, 1979; Reich, 1978), and they will, no doubt, be echoed in student discussions.

1. Amoralist: This attitude denies the existence of ethics and rejects the possibility of there being valid ethical principles or ways to establishing them.

2. Relativist: Relativism stresses that values and principles change constantly; they lack consistency and hence vary in use with time and place.

3. Intuitionist: The claim here is that ethical knowledge comes from intuition; it does not have to be established by reason or rational discourse.

4. Utilitarian: People with this attitude sometimes refer to their views as "practical"; ethics to them relates to the consequences of ethical action, not to principles that establish action. For example, utilitarians will tend to judge an issue according to the extent to which it will enhance or detract from the well-being the "common good"--of humankind and nature.

5. Person focused: This attitude asserts that the integrity of a person and respect for human dignity are the ultimate, and sometimes sole, criteria for ethical decisions.

6. External-authority focused: This attitude acknowledges a predetermined, unchanging purpose for events; the nature of the purpose will determine judgments on ethical issues, a process often described as obeying natural law.

7. Process-focused: This attitude depicts ethical analysis as a fundamental determinant of ethical judgment; it is an attitude parallel to that of many scientists in that ethics and scientific methods require commitment to rational discourse. "Scientific method is a form of rational inquiry into facts, empirical matters. It asks: What is the

> case, and how can we best explain it? Ethics is a form of rational inquiry into right and wrong action and good and bad character. It asks: What ought we to do or be, and what justifies our answers to such questions? [Both] methods insist on rigor and on following...rules for rational inquiry and discourse" (BSCS, 1988).

B. Moral codes. The slightest acquaintance with any of the world's religions provides an illustration of a moral code, a set of assumptions and propositions that describes the purpose of existence and provides criteria to which ethical judgments conform. The source of a religious code is revelation, often converted into a written form as with the Christian Bible, the Hebrew Torah, or the Islamic Koran.

Moral codes also can relate to other sources. Some, such as atheism and various forms of humanism, are based on the denial of the existence of an external moral purpose. They are sometimes expressed through the person-focused attitudes referred to earlier. Other codes such as communism, socialism, and the variety of forms of capitalism have their sources in political and economic ideology.

Some codes are related to professions. The most obvious example--and one very relevant to biotechnology--is the Hippocratic Oath, sworn by physicians in many countries. The oath assumes the primacy of the individual patient and protection of his or her privacy in medical conduct.

Even biology has been the source of moral codes. In Europe and North America, particularly during the latter part of the last century and the early years of this, the theory of evolution has provided the source for a variety of codes. Possibly the best known were those loosely grouped under the term Social Darwinism, which postulated that inequalities based on inborn differences and competition were norms on which good conduct should be based. Social Darwinism had considerable influence on politics, business, and education at the time.

Other codes based on biology are conceived in a broader frame work. Schaefer (1987), for example, postulates twelve principles of life, including concepts such as adaptation, individuality, and entropy reduction, which he suggests should guide human behavior and ethical decision making.

People tend to examine ethical issues from the standpoint of a variety of moral codes, and this holds true for ethical issues related to biotechnology. The difficulty arises when one tries to reconcile these various codes in order that judgments can be made for common actions. The wider the variety of codes, the greater the problem, which, of course, explains the difficulty of dealing with ethical issues on an international scale. The reconciliation of diverse ethical codes is not, however, impossible, as was shown by the work of the President's Commission for

the Study of Ethical Problems in Medicine and Biomedical Research (1983), which functioned in the United States from 1977 to 1983. The commission consisted of a group of people from diverse social, professional, and religious backgrounds. They consulted a wide range of experts such as scientists, philosophers, and religious leaders and were able to propose a set of principles (a code) for dealing with ethical issues of human genetic engineering. These were:

1. Confidentiality: that we respect the privacy of a person and not disclose personal information (including genetic information) obtained in confidence.

2. Autonomy: that we acknowledge that a person has the right to make decisions that affect him or her and should have the right not to participate in any action affecting him or her without giving consent freely and voluntarily. Those who are not competent to do this, such as children and the mentally impaired, should be given special protection.

3. Knowledge: that a person be given sufficient information to make any informed decisions. This rests on the primary value that people are always told the truth.

4. Beneficence: that we should do no harm to others unless the benefits clearly outweigh possible harms.

5. Justice: that we should treat people fairly and equally.

Although this list demonstrates that people can agree on a set of ethical principles, there is still opportunity for a variety of interpretations of the principles. That is, there is opportunity for ethical dilemmas, which arise when there is substantive support for the primacy of more than one ethical principle. The prospects for such dilemmas signals to us both the level of possibility and the inevitable difficulties that ethical issues in biotechnology can--and do--produce.

C. Strategies for ethical analysis. Analysis of ethical issues, particularly on a social scale, requires rational standards and the use of rigorous methods. This, essentially, is the expression of a process-focused attitude towards ethics, which attempts to minimize, if not eliminate, the influence of preconceptions and unsupported assertions. Others--especially those who hold attitudes that are utilitarian, person-focused, or focused on external authority--would, no doubt, also argue the need for a rigorous method, but within the tenets of the framework set by their attitudes. Theirs is a less open approach.

To comprehend the variety and nature of ethical methods would require that students take a course in ethics. This would be valuable as long as it was relevant to the issues they would be dealing with in their science course. It is unlikely, however, that most students will take a course in ethics, and science educators have therefore developed mechanisms that teach the central elements of ethical analysis in the context of a science course. The example provided below was adapted by Dr. W.V. Mayer and is based on the work of the Biological Sciences Curriculum Study in the U.S.A. (BSCS, 1988). The method is based on four processes--interpretation, analysis, argument, and critique--and consists of nine steps taken in sequence. In this description the method is applied to the following issue: "Should genetically engineered bacteria be used in agriculture to minimize frost damage to plants?"

Step 1. Identify value-related concerns (interpretation)

This step is designed to distinguish factual dimensions of science from value-related ones. The factual dimensions are descriptive in character. They may concern observations, data, hypotheses, and theories. The value-related dimensions concern whether some aspect of science is a right or wrong thing to do.

> The students should, for example, acknowledge that "genetically engineered bacteria" is a factual dimension and "should they be used in agriculture?" is a value-related dimension. The students also should be clear that they are not, at this time, dealing with the issues "Should genetically engineered bacteria be produced?" or "Is it right or wrong to undertake genetic engineering?" They have to ignore such issues or decide on them and use the decisions they come to as value-related elements in dealing with the focal issue.

Step 2. Identifying how the value-related concerns are related to science (analysis)

Here students analyze the issue at hand to identify the specific aspects of science that are the source of an ethical concern.

> For example, the issue of using genetically engineered bacteria in agriculture is likely to be related to ecological and evolutionary considerations. Will the bacteria spread to other areas, and will they replace the more prevalent, wild-type *bacteria that do not protect plants against frost damage? Will* the spread of the genetically engineered bacteria alter the effects of natural selection on plants? A judgment to support the use of the bacteria would assume that such changes will have little or no significance. A judgment that use of the bacteria is wrong

Students frequently have trouble identifying which aspects of science are the source of their ethical concerns. This may be due to incomplete knowledge of scientific information. It is here that many discover that their concerns are based on misinformation or misinterpretation.

> For example, students may have an image of genetic engineering *per se* as being good or bad. They will need to work out whether that image is valid and whether it is relevant to the issue being considered.

Step 3. Trace the implications (analysis)

This process focuses on moral values (codes) themselves to demonstrate that they have implications for how we act. Students frequently are unaware of moral values in their lives. In part, this results because we are not always clear about what moral values are relevant or important in complex situations; moral values can have more than one implication. Steps one and two are designed to clarify this problem, but the implications of moral values may not be clear to students. Patience is required while students learn to develop the skills of tracing implications of moral values. Class discussion is critical here. Students should identify the implications that follow from the values they have identified. That is, they should state what ought or ought not to be done about the aspect of science that is the source of concern.

> With respect to genetically engineered bacteria, for example, students may adhere--even subconsciously--to a moral code that considers natural situations sacrosanct. The implication of moral values would, presumably, be that the bacteria not be used in agriculture. Other students may consider human supremacy expressed in technology as morally good. The implication of that moral position is that use of the bacteria is acceptable.

Step 4. Explain why the implications follow from the stated value (argument)

Here, the concern is with the need to give reasons for the implications for moral values related to science. One must differentiate between mere opinions, the ability to assert implications without being able to explain them, and clear statements of the reasons that explain why the implications developed in step three follow from the elements identified and discussed in steps one and two. Unexplained assertions are no more admissable in ethical analysis than are unsupported hypotheses in science.

Through such considerations, students will experience the special intellectual skill of making arguments for a moral point of view expressed as an implication derived from a moral value. Students should come to appreciate the care and precision that arguments require, and to appreciate that argument is a process that benefitsfrom group discussion and the insights and views of classmates. In this step, it is imperative to involve as many students as feasible in discussion.

At this step there is a strong and natural tendency among students to raise questions about, and to criticize or disagree with, the responses of other students. This needs to be controlled. It is still too soon for a critique, because the process of placing a representative range of reasonable points of view before the group has not yet been completed.

Step 5. Identifying alternative values (analysis)

Fairness in ethical analysis requires that students learn about and take seriously the full range and variety of moral values that people regard as important or relevant. One now calls on students who have expressed disagreement with what has been said so far to give them an opportunity to express their views and have others analyze them. This step especially emphasizes the participatory nature of

Possible comments that may emerge from the discussion include the argument that a particular proposition rests on an external moral code such as that of a particular religion. In such a case, the teacher would need to draw the distinction between revealed opinion and scientifically acquired knowledge.

Someone might contend that the significance of "natural" as a decisive moral criterion rests on the proposition that the complexity of ecological relations makes it difficult to determine the effects of interference. Alternatively, the idea of risk as a statistical entity might be used to argue a less decisive relation. Such explanations get nearer to valid implications.

doing ethics and promotes tolerance and respect for moral values other than one's own.

Steps 6 - 8. Recapitulation (analysis and argument)

Having identified alternative values in step five, students subject them to the routines of previous steps: identifying the sources in science related to them (step 6); tracing their implications (step 7); and developing an explanation for each one (step 8).

Step 9. Critique

This is the culminating activity in which, finally, the students draw conclusions. If time is short, critique could follow step four, but the quality of the work will be diminished.

By step 9, students should have determined a number of moral judgments (values), each described by a clear statement. In addition, each judgment should be supported by the reasons for its being considered important and relevant, with the scientific sources that occasioned the moral concern clearly identified. The implications should be drawn carefully from the value, and the reasons for these implications argued succinctly.

With a class, it is best to consider one value and its attendant supporting comments at a time. The students should be helped to examine this value through orderly discussion, bearing in mind that random and contentious argument is pointless and that successful critiques tend to follow one or more of the following propositions.

1. The statement of the moral value is unclear. The attempt to clarify the value may show that the original implication does not follow from the value, in which case one needs to return to step one and focus on a reformulation.

2. There are no empirical grounds for being concerned with the moral values and its implications, because the supposed source of the ethical concern in science does not exist. In this case, one needs to return to step two to begin again the process of justification.

3. The implication does not follow from the stated moral value, because the reasons that have been identified for drawing the implication do not together support it. This requires a return to step two for justification.

4. One or more of the reasons offered in support of the justification are not valid. Conclusions about implications do not relate to the reasons given. If this is the case, a return to step four is required to start the process of justification over again.

5. The reasons can be shown to support contradictory implications. If this is the case, step five must again be initiated.

At this stage, students should be encouraged to accept or reject a position. They should be willing to give up an implication, for example, if any one of the reasons for it is false, unfounded, or confused. They should realize that the critique ends if the reasons at the heart of the argument are unsupported. They also should understand that if more than one conclusion can be drawn from a value, their line of reasoning yields contradictory conclusions and hence cannot help in decision making.

7. Determination of Policy.

Policy determination leading to eventual action is, in effect, the ultimate purpose of all that has been written about so far. Its procedures follow those outlined for dealing with social implications, as shown in table 1, but the central consideration is: now that we have decided on the nature of the problem and have decided on a solution--or possible solutions--what are we going to do about it? This requires a plan of action--a policy. If, for example, the issue at hand is the production of a genetically engineered pharmaceutical product, and we have concluded that benefits outweigh risks and that it meets with general moral approval, the policy might be concerned with the location, construction, and operation of a pharmaceutical industry. If the issue had to do with the use of a new

technique for genetic screening, for example, the policy no doubt would address incorporation of the technique into genetic or medical procedure. (if the technique had been approved), or it might address legal requirements to regulate or prevent use of the technique if there were doubts about its scientific or moral validity.

Policy determination uses the same intellectual techniques and selects from methods such as those referred to in the previous section. The particular mix of ideas and procedures adopted will vary with the type of policy being devised, but, as in dealing with the social implications of science, the essential features are a careful, methodical, and rational approach; an appreciation of the distinction between facts and values; and a pervasive sense of realism--what some have called the art of the possible.

A. Areas of policy determination. Policy determination in biotechnology can be divided broadly into personal and social categories. *Personal* policies involve matters that have an intimate relevance to people as individuals. Policies related to genetic screening and diagnosis and gene therapy are often of this nature. Action that arises from biotechnology in these fields frequently requires, at least in part, personal rather than collective decisions.

Personal policies can be linked to social policies when, for example, general regulations are determined for human experimentation by society acting collectively, usually through a professional body or the government. Unless this includes the obligation to take part, however, such a social policy will have within it a personal-oriented policy for dealing with the issue of individual choice.

The range within the category of *social* policies is considerable. The following, adapted from a report on the impact of biotechnology in Europe (Yoxon, 1987), provides a review of some of the major social policies.

1. *Innovation policies* concerned with stimulating or regulating new developments in biotechnology. Examples include the provision of tax concessions to entrepreneurs, and directive industrial planning by governments.

2. *Research policies*, which relate to such issues as maintaining the independence of scientific research as an open source of new knowledge, regulating research to meet specified economic and industrial needs, or moral considerations.

3. *Patent policies*, which are concerned with ownership of discoveries and inventions.

4. *Safety policies*, which cover issues such as workplace safety, product

safety, and environmental pollution.

5. *Economic policies*, which are particularly concerned with financing biotechnology in relation to the overall needs and available budgets of a society. Examples include price-support grants to encourage the adoption of biotechnologically produced organisms in agriculture and the reallocation of financial support from clinical to preventive medicine to encourage the use of screening and diagnostic methods made possible by developments in biotechnology.

6. *Trade and aid policies*, which relate to transactions within and between countries. These policies address, for example, issues such as the provision of aid to developing countries in the form of access to research facilities, the development of new industries, or the provisions of ready-made products.

7. *Social policies*, which affect social structure and culture. They cover topics such as changing employment patterns arising from the development of biotechnology industries, changes in population size and composition arising, for example, from people living longer as a result of biotechnological developments in medicine, and moral issues arising, for example, from religious attitudes towards biotechnology.

B. When is policy determination appropriate? Four criteria are relevant when deciding whether policies are appropriate at a particular time (BSCS, 1988):

1. Is the situation urgent? Is there an immediate risk of serious, far-reaching, and irreversible harm? "Immediate" means that the harm can, on reliable scientific grounds, be expected to happen in the near future. We therefore have to think about it and have to do something about it now. "Serious" means that the risk involves potentially grave consequences. These include such things as increased morbidity and mortality to plant, animal, or human life; damage to the ecosystem or environment; or considerable economic loss. "Serious" also means that important values such as freedom are threatened. "Far-reaching" means that the impact of the consequences may be widespread. Considerable numbers of individuals or groups, large geographic areas, and industrial sectors may be adversely affected by the consequences. "Irreversible" means that the adverse consequences are expected to be permanent.

2. Are there scientifically valid means available to prevent, avoid, or reduce the risk of such harm?

3. Is the policy conducive to a broad-based societal consensus? This means that people, corporations, and governmental entities will not perceive the policy as being directly at odds with their own values and concerns. This is a relevant test, for example, both for public policy that *permits* certain types of genetic engineering and for policy that *prohibits* certain types of genetic engineering. Ideally, many will see public policy as serving or at least as consistent with and even furthering their values.

4. Is the public policy feasible? For a policy to be effective, we should expect that most people, corporations, and government entities will abide by it. The policy must be enforceable. It is unlikely that a public policy will be enforceable for those individuals, corporations, or governmental entities whose values are violated by the policy. Policy also is unlikely to be enforceable if people, corporations, or governmental entities in large numbers are simply not going to abide by it, as was the case with Prohibition in the United States.

C. Who should determine policy? Those who set policy invariably confront a range of factors such as scientific and technological data, social and economic issues, and ethical considerations. Decisions, to some measure, reflect the people who make them. A scientist, economist, and theologian, for example, would bring different perspectives to bear on an issue and those perspectives will reflect their professional knowledge and experience, and their personalities, which may not necessarily relate to their respective professions. Thus, in drawing up a policy, a key question is: Who should do it? This, in turn, raises three particular issues: the role of the expert (and what we mean by an expert); the efficiency of undertaking the task; and the likelihood that the policy will be accepted and implemented.

1. *Expertise*. Knowledge and understanding are central to policy determination. Clearly, determination of biotechnology-related policies requires scientific, technological, medical, agricultural, or other expertise. This scientific and technologic expertise must, however, be complemented by expertise in fields such as ethics, law, or economics. Such expertise will come from appropriately trained professional people.

   There is, however, another type of expertise required that is not to do with a profession nor with depth of knowledge in a particular specialty. This is the expertise of people--very often generalists--who can synthesize and evaluate a variety of evidence and make decisions about them uninfluenced by specialized, and hence narrow, viewpoints. A yet further type of useful expertise is paradoxically that of

"inexperts," particularly if they are representative of groups of people the policy will affect. Such expertise may come from representative lay people or be acquired through opinion polls or comparable methods.

The range of experts required will vary according to the policy being developed. In drawing up the policy, each expert has the role of providing evidence and discussing the specific aspect of the policy with which he or she is concerned. This may occur directly and personally at meetings, or indirectly through reports or other nonpersonal forms of communication.

2. *Efficiency* of policy making relates to the structures within which the people concerned work. The structure should provide sufficient input of information, evidence, and discussion while enabling decisions to be made effectively. The composition of the structure will vary according to the topic of the policy under consideration. For a policy whose focus is an ethical issue, the most effective structure likely would involve a broadly representative group of people aiming at a consensus, or the provision of alternatives for individual decisions. A policy aimed at designing a biotechnological industry will require, however, a more hierarchical structure. This would involve a few people making final decisions based on consultation with an appropriate range of experts.

Efficiency also will depend on the structure of decision making. This should ensure that each relevant issue is considered in turn and related to decisions as they are required. If possible, decisions should flow from the evidence provided. It is only when opinion alone remains that more subjective decision-making methods such as voting, developing a consensus, or accepting a number of alternative conclusions should be employed.

Consideration of acceptability and implementation will inevitably broaden the remit of a specific task of policy making and require it to be linked to policies in associated areas. Such relationships are shown in table 2, which is based on studies in Europe. The table lists a number of domains that are, or are likely to be, affected by developments in biotechnology; opposite them are listed some of the effects that may arise from them. Policy making concerned with any of the issues outlined on the left-hand side of the table will need to take into account the possible effects listed on the right.

A review of table 2 reveals a final, but fundamental, attribute of policy making: only rarely will policies that affect social implications be narrowly circumscribed. Such policies, rather, will be influenced by policies and events elsewhere and by policies that come about as time changes. Certainty and stability, therefore, are rare characteristics of policies that address the social implications of fields such as biotechnology.

The various characteristics of policy making can be incorporated, together with other aspects of dealing with the social implications of biotechnology, into simulated case studies. These provide students with an experience that closely mirrors real-life issues involving biotechnology. Examples are provided in the next chapter.

Table 2. Types of development arising from biotechnology in different domains, and their possible effects (Yoxon, 1987).

DOMAIN	POSSIBLE EFFECTS
Medicine	
Increased availability of treatment, e.g., biotechnological production of insulin	Major diseases such as heart disease and diabetes managed more efficiently; overall impact on mortality arguable.
New forms of diagnosis, e.g., DNA probes	More rapid intervention; more reproductive advice possible; risks of anxiety/stigma if done carelessly.
Agriculture	
Substitution for existing agricultural products. New forms of agricultural production, e.g., enzyme technology for sweetness, instead of sugar-beet production.	Surplus capacity; loss of markets for some farmers if diversification fails. Shift away from the land; regional relocation; more industrialized, less seasonal production; jobs displaced to another sector.
Environmental control	
Pollution, e.g., bacterial degradation	New solutions for major waste problems; some treatment processes cheaper; possible creation of new jobs.
Biotechnology process effluent	Volume of "traditional" effluent increased; new problems created, such as plasmid DNA.

Work	Diversification into (uptake of) biotechnology strengthens a general trend to flexible production.
	More processes based on fermentation, risks of explosion, toxic gases, and liquids reduced; novel problems of fermentation safety arise.
	More shift work in areas where continuous processes now possible. Some areas of craft production undermined, e.g., in food industry. Some additional, highly specialized jobs in new, small firms.
General culture	Changes in patent law influence what counts as private property. More genetic data kept on file, much of it of debatable value.
	Attitudes to recycling and the use of renewable resources change. Role of the universities transformed. Ideology of research-based entrepreneurship and production stronger.

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Chapter 4

SUGGESTIONS FOR TEACHING BIOTECHNOLOGY*

Introduction

This chapter contains teaching examples for the fields of biotechnology described in detail in chapter two. The suggestions have been conceived with the upper grades of a ten-grade compulsory school system in mind, although some (for example, making yogurt and sauerkraut) were designed for lower grades. The focus is on practical experiments and case studies. The experiments are intended to do justice to the nature of biotechnology as a practical, experimental discipline. The students should be encouraged to overcome their inhibitions in dealing with microorganisms or biological agents such as enzymes or DNA while learning careful handling procedures in clean, often sterile conditions. It is to be hoped that when the students are called upon to make decisions in later life, this experience will have provided them with a better emotional basis for making judgments than fear alone. The same precision demanded in experimentation is required in the ethical analysis of problems resulting from the application of biotechnology (see Chapter 3) if students are to realize that there can be more than one reasonable solution to a moral problem. This realization, and the recognition that ethical analysis may lead to diametrically opposed points of view, can lead to more tolerance in dealing with opinions that conflict with one's own.

The lessons included here need not be taught as a complete unit in a separate biotechnology course, nor is it necessary to follow them in the given sequence. They may be used as appropriate within conventional science subjects up to grade 10. Biology is the most obviously relevant subject, but health and environmental education, if taught separately from biology, would also lend themselves to biotechnical themes. The list below suggests areas in which the examples would be appropriate. The incorporation of biotechnical topics demonstrates to students that biology has become an industrial science.

1. Cytology

Investigation A.	**The Division of Microorganisms into Large Groups by Observing Colonies**
Investigation C.	**Making Fixed-Slide Preparations**
Investigation D.	**Making an India-Ink Preparation**

*This chapter was written by H. Bayrhuber, E.R. Lucius, Ch. Gliesche, Ch. Labahn-Lucius, U. Nellen, and R. Westphal. Jane C. Riley and Gillian Horton-Kruger were the translators and Erika-Anna Kolaczinski was the illustrator.

2. *Ecology and Environmental Education*

Investigation B.	**Enrichment of Wild Yeast Strains**
Investigation F.	**Tracing Soil Bacteria that Decompose Urea**
Investigation M.	**The Microbial Decomposition of Cigarette Paper**

3. *Physiology and Nutritional Education*

Investigation J.	**How are Yogurt and Sauerkraut Produced?**
Investigation K.	**The Formation of Lactic Acid in Sourdough**
Investigation L.	**Wine from Juice, Vinegar from Wine**
Investigation N.	**Why Does Apple Juice Gel When It is Boiled?**
Investigation O.	**Pectinase--An Enzyme that Decomposes Pectin**
Investigation P.	**Industrial Uses of Pectinase**
Investigation Q.	**The Splitting of Lactose from Milk or Whey by Immobilized Lactase**

4. *Health Education*

Investigation E.	**The Safe Microscopy of Mold Using the Petri-slide Technique**
Investigation G.	**Demonstrating Production of an Antibiotic**
Investigation H.	**Demonstrating the Effect of Streptomycin in the Small-Disc Test**
Investigation I.	**Demonstrating the Presence of Bactericidal Substances**

5. *Developmental Biology and Breeding*

Investigation R.	***In Vitro* Culture in the Ornamental Usambara Violet**
Investigation S.	**The Cultivation of Gerbera: More Economical by *In Vitro* Culture**

6. *Genetics*

Investigation T.	**Isolating DNA from Sweetbread**
Investigation U.	**Conjugation in Bacteria**

7. *Biotechnology and Ethics: Selected Case Studies*

Case Study A.	**Microbiological Aid for Villages**
Case Study B.	**An Enquiry into the Proposal to Map and Sequence the Human Genome**
Case Study C.	**Conflicting Assessment of DNA Analysis in Prenatal Diagnosis**

The experiments are amply illustrated to allow teachers to follow the steps quickly and easily, and the lists of required equipment and materials show at a glance what is needed to conduct the experiments. The lessons assume familiarity with the basic techniques of microbiology; teachers whose training did not include those techniques should arrange for in-service programs. In view of the development of biology into a science with industrial application, we regard the appropriate skills as indispensable. In this connection we recommend two books:

Microbiological Techniques in Biology Teaching, by Ronald Westphal (1989), which describes the techniques developed at the IPN, and available there. Write to: IPN, Kiel University, 2300 Kiel 1, F.R.G.

Practical Microbiology and Biotechnology for Schools, by Paul Wymer; 30 photocopiable pupil's workcards and teachers' guide (1987), available from the National Centre for School Biotechnology, University of Reading, London Road, Reading RG1 5AQ.

Microbial Systems

For many students age 7 to 15, microorganism are synonymous with pathogens for which physicians prescribe antibiotics. Many students are unaware that most microbes are completely harmless and that a number of them are extremely useful. Students should learn to be discerning with respect to the human uses of bacteria and fungi and to attempts to combat harmful organisms. The fungi among the microorganisms are divided into two large groups according to the morphology of their colonies (yeasts and filamentous fungi) and are distinguished from *Streptomyces*, which are bacteria.

The production of antibiotics, demonstrated in this section, is a notable achievement of microorganisms. In addition, this section includes a procedure for the safe microscopy of mold. Experiments with other substances that inhibit the growth of microorganisms (for example, heavy metals from coins) also are important and are included in this section.

The various functions of microorganisms that are used industrially should be made clear by screening procedures.

1. The Observation of Colonies of Different Microorganisms with the Naked Eye.

When students are introduced to microorganisms they should learn to categorize colonies of these organisms in four different groups according to macroscopic structure: molds, yeasts, protists, and bacteria, including *Streptomyces*. One cannot generally accomplish an exact classification of a yeast or bacterium with the naked eye.

The students can consider the great economic importance of members of these various groups while observing the colonies. Many students may be unaware that *Streptomyces* are used for the industrial production of antibiotics such as streptomycin or tetracycline, and also can be used to produce dyes.

The similarity of *Streptomyces* to fungi is restricted to their similar morphology, that is, their ability to form mycelia. *Streptomyces* are, however, bacteria. They do not possess proper nuclei; neither do they possess cell organelles such as mitochondria, and they have a cell wall that is made of lipids, carbohydrates, and protein. *Streptomyces* occur in soil; in fact they give the soil in woods its typical "earthy" smell. The following investigation describes the cultivation and classification of mold, yeast, and bacteria, including *Streptomyces*. The concluding investigation addresses the enrichment of wild yeast, the cultures of which are often colored.

Investigation A. **The Division of Microorganisms into Large Groups by Observing Colonies (by C. Gliesche & E.R. Lucius)**

Prerequisite Biological Knowledge

awareness that microorganisms exist

Equipment

1 bunsen burner
1 inoculation loop
1 conical flask, 250 ml

Materials

100 ml basal agar medium (see appendix)
3 petri dishes
1 felt-tip pen
50 ml basal broth medium (see appendix)
cultures of the following microorganisms, incubated overnight:
Penicillium roqueforti (DSM-No. 1079)
Rhodotorula rubra (DSM-No. 70403)
Streptomyces griseus (DSM-No. 40236, ATCC 23345)

Bacillus subtilis (DSM-No. 1079, ATCC 6051)

Time Requirements

preparation of over-night cultures in basal broth medium: 45 minutes
incubation of over-night cultures: 24 hours
inoculation: 15 minutes
incubation: 6 days

Procedure

1. On the day before the investigation, prepare cultures of the four species of microorganisms; incubate overnight.
2. Prepare 100 ml of basal agar medium (3 agar plates).
3. Divide the plates into 4 sections on the underside, using a felt-tip pen.
4. Inoculate each of the 4 sectors at one point with one of the 4 types of microorganism.
5. Incubate the plates at 30°C for 6 days.
6. Identify the colonies according to the diagram below (figure 2).

Explanations and Suggestions

A - Molds:

Soft, stringy colony; colorless mycelium also grows below the surface of agar medium. Aerial mycelium of *Penicillium roqueforti* usually with blue-green spores (spores of other fungi are also colored brown/yellow or black). Usual diameter of a single colony: often more than 10 mm.

B - Yeasts or Bacteria (other than *Streptomyces*):

Shiny-matt or slimy colonies often above the surface of the agar. Color of the colonies often grey/yellow (usually bacteria, grey colonies of *Bacillus subtilis*) or grey/red/orange/yellow/brown (usually yeasts, red colonies of *Rhodotorula rubra*). Usual diameter of a single colony: often less than 10 mm.

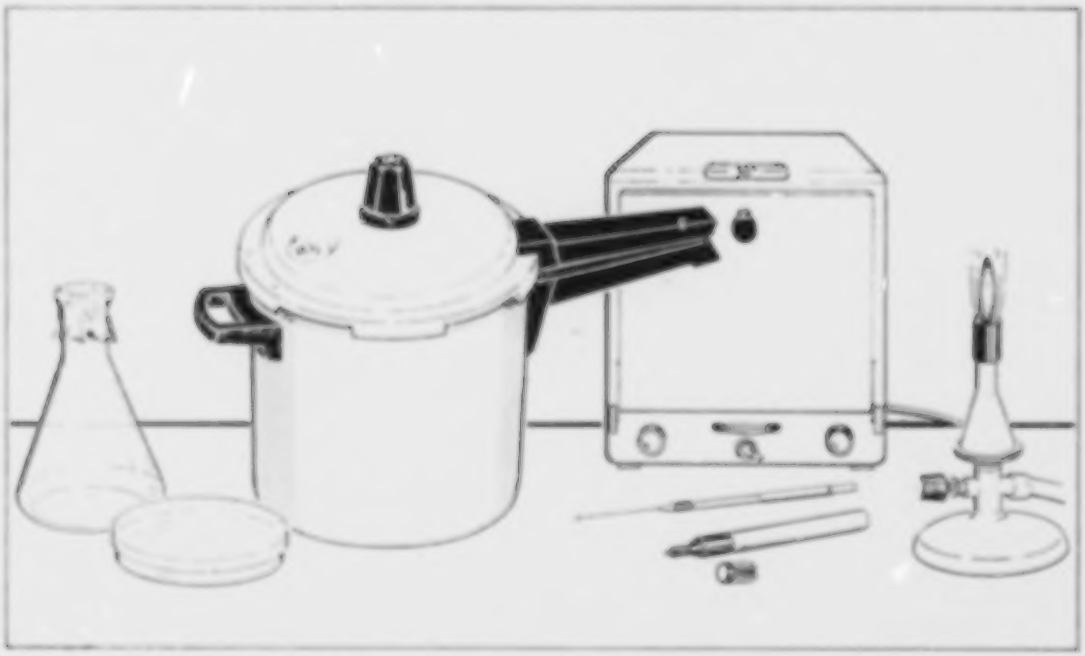

Figure 1. Required equipment.

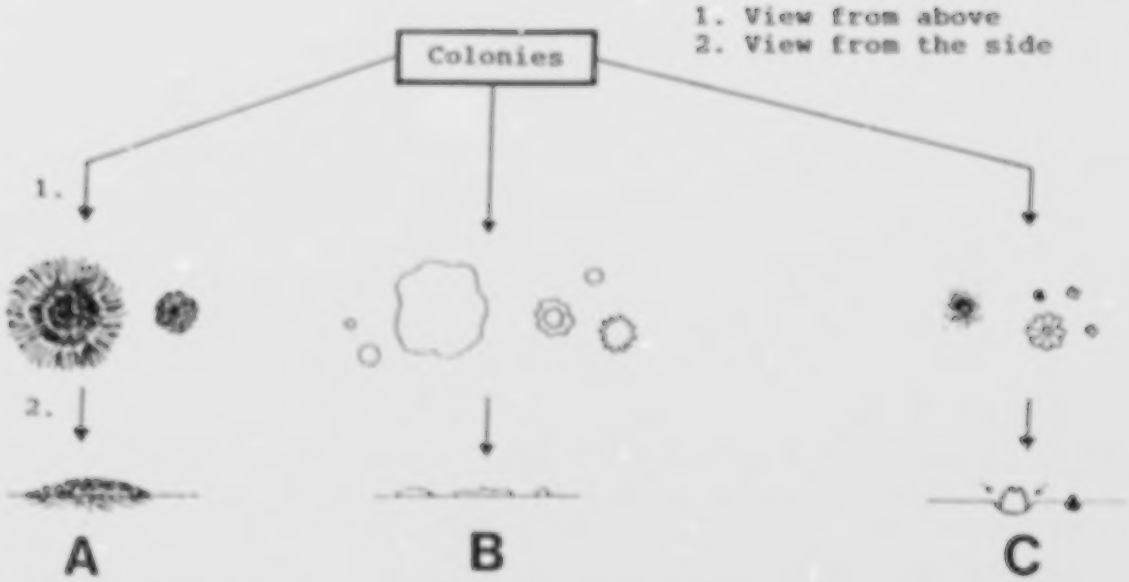

Figure 2. From colonies to macroscopic division of microorganisms.

C - *Streptomyces*

Earthy smell from the petri dish. Truncated, round, single colony that grows in the shape of a lens in the nutrient. Aerial mycelium (after incubating 6 days) is often colored; *Streptomyces griseus* produces grey aerial mycelium (some other *Streptomyces* stain the surrounding agar brown). Usual diameter of a single colony: often less than 3 mm.

Investigation B. **Enrichment of Wild Yeast Strains (after R. Westphal, 1989)**

Yeast cultures derived from natural isolates can be produced easily within closed petri dishes for demonstration purposes. For safety reasons, however, they should not be used for further experiments.

Prerequisite Biological Knowledge

the ecological significance of wild yeasts as decomposers

Equipment

petri dishes

Materials

malt agar medium (see appendix)
an unwashed apple, or another piece of fruit with sugar content

Time Requirements

45 minutes

Procedure

1. Prepare malt agar plates according to the instructions in the appendix.
2. Roll a piece of unwashed fruit across the surface of the chilled agar; close the dishes immediately.
3. Incubate the plates for a few days at 30°C. An array of yeast types, some brilliantly colored, can be observed, along with mold cultures that can be recognized by their cotton-like texture.

Figure 3. Petri dishes.

Figure 4. Cultures of assorted yeast types from an apple skin.

2. *The Observation of Microorganisms Under the Microscope.*

Students' use of bacteria should not be limited to the inoculation and incubation of agar plates, however important a procedure this may be in lessons. Students quickly lose interest in merely observing colonies or holes in the bacterial lawn, which very often appear similar. Teachers should therefore use various methods, especially where microbial systems are concerned. Observation using a microscope is one good method. Students are extremely interested to find out what the ubiquitous bacteria look like; they never see bacteria as individual organisms with the naked eye.

The investigations in this section present techniques for the fixation and microscopic observation of bacteria. Westphal (1989) also describes further dyeing techniques for use in schools--for example, dyeing with methylene blue, and gram staining. (Westphal's publication is available through IPN, 2300 Kiel, FR Germany).

The cultivation and observation of mold is a common procedure in schools. Students know that some molds spoil food, and that others can be used in the production of food (for example, camembert, Indonesian bean cake tempeh, soja bean cheese sufur). There are considerable safety risks, however, in the open microscopy of mold. A student who accidentally coughs into a spore culture can propel large numbers of spores into the air. Certain types such as *Aspergillus niger* can infect the respiratory tract, which can be fatal for those whose immune system has been weakened (Staib, 1985). The petri slide procedure for safe microscopy of mold (Lucius, Fries 1990) avoids these risks. From a purely technical point of view, this procedure also produces far more satisfactory results than the observation of mold in a normal petri dish through a microscope.

Investigation C. Making Fixed-Slide Preparations (after R. Westphal, 1989)

Because it is difficult to focus sharply on living bacteria, they are almost always observed in fixed preparations. Furthermore, fixed bacteria can be stained easily.

Prerequisite Biological Knowledge

know the difference between bacteria and yeasts
ability to use a microscope

Equipment

1 microscope slide

1 cover slip
1 eye dropper
1 bunsen burner

Materials

ethanol

Time Requirements

30 minutes

Procedure

1. Remove grease carefully from a microscope slide with a lint-free towel or a piece of tissue soaked in ethanol.
2. Place a drop of bacteria or yeast suspension in the middle of the microscope slide. The drop should flow out evenly and must not remain in globular form (figure 6).
3. Place a cover slip on the microscope slide at an angle of 45° so that the solution is collected in the space between the slide and slip and held by the properties of adhesion and cohesion (figure 7).
4. Push the cover slip evenly across the entire surface of the microscope slide. This spreads the suspension across the slide, and the film of liquid becomes thinner (figure 8).
5. Allow the smear to dry.
6. Fix the bacteria to the slide by briefly heating the slide in a flame. This can be done in one of two ways: with a low flame such as the pilot flame of a bunsen burner and with the coated side of the slide oriented downwards, or with the high flame of a bunsen burner and the coated side of the slide up. Pass the slide through the flame three times at a speed of roughly 30 cm per second.

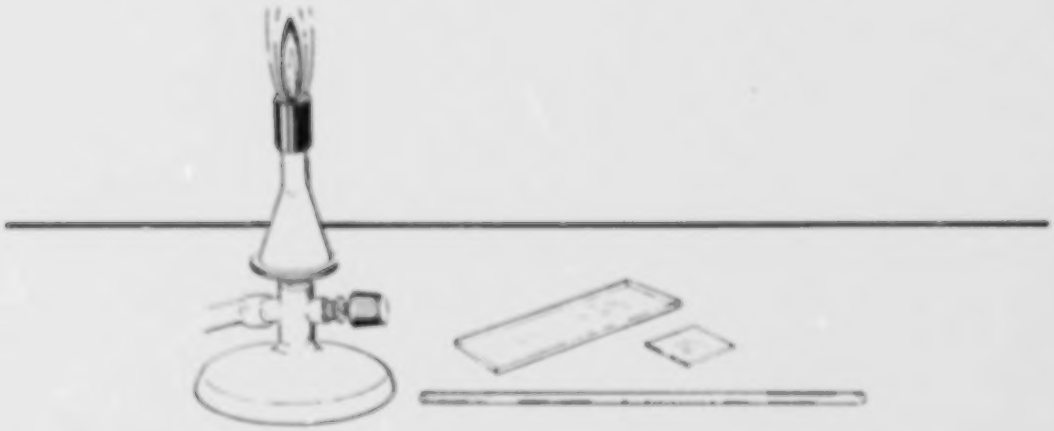

Figure 5. Required equipment.

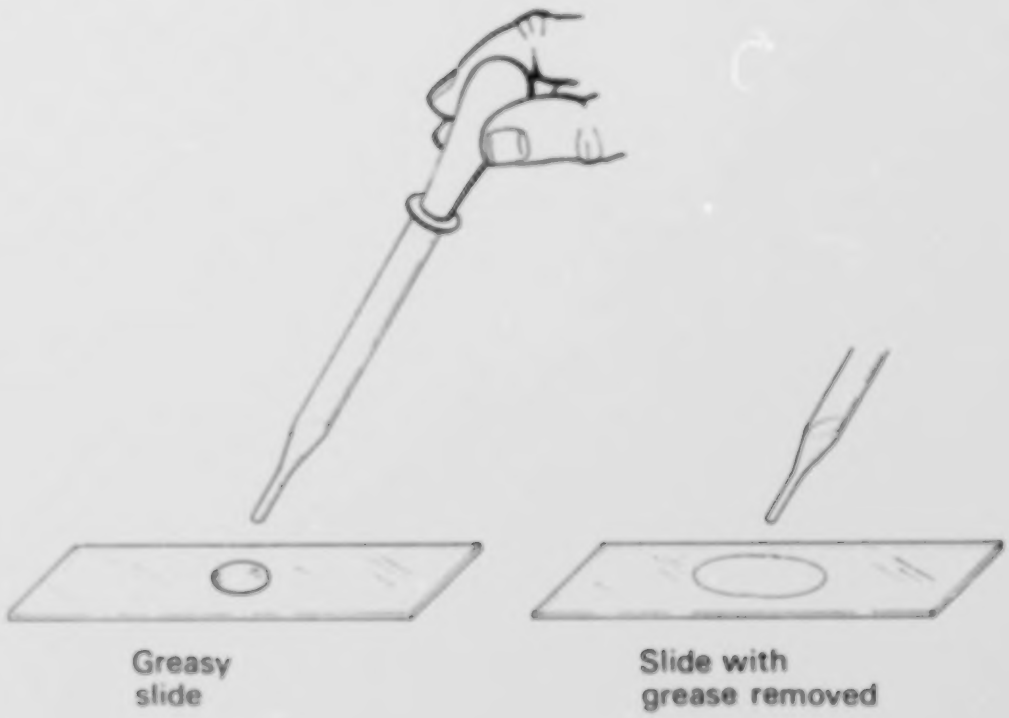

Figure 6. Spreading a drop of liquid on a lubricated and on a non-lubricated microscope slide.

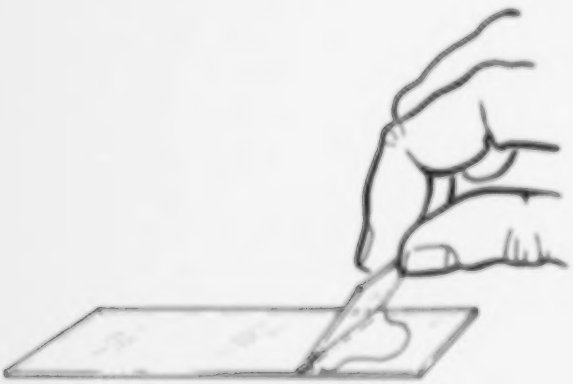

Figure 7. Placing a cover slide on a microscope slide.

Figure 8. Proper movement for spreading a drop of liquid.

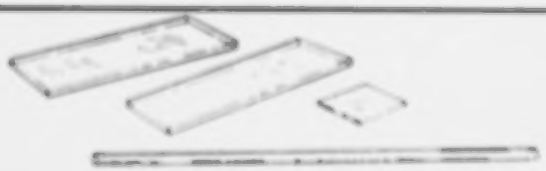

Figure 9. Required equipment.

Explanations and Suggestions

Steps 1 and 2. The suspension eventually will flow back together, even when only traces of grease are present on the slide. This not only lengthens the drying time, but allows thick layers of bacteria to develop, as well. Consequently, it may no longer be possible to observe individual microorganisms. NOTE: If attempts to remove grease from the slide are unsuccessful, use a drop of extremely diluted bacterial suspension--allowed to air dry--in place of the smear technique. For younger students, this method is easier than executing a smear with the delicate cover slip (figure 8).

Step 3. It is important to pull and not push the suspension across the slide with the cover slip to ensure that the thickness of the coating decreases evenly.

Step 4. This drying step must not be accelerated by heating. Bacterial structure changes when bacteria are heated in water.

Step 5. The first method is recommended. Because of the poor conducting qualities of glass, it is difficult to estimate the effect of heating on the bacteria with the second method. Organisms and the protein coagulated in the cells by heating adhere to the slide surface.

Investigation D. **Making an India-Ink Preparation (after R. Westphal, 1989)**

An easy way to improve the contrast of microscopic examination is to stain the background uniformly black by the use of India ink. Only the organisms are illuminated in the microscope, and they appear in bright contrast to the background. This process is especially suitable for beginners because it leads to clearly recognizable results and allows organisms and dust particles to be distinguished easily. Simple school microscopes are satisfactory for the observation of these preparations.

Prerequisite Biological Knowledge

ability to use a microscope
knowledge of the difference between bacteria and yeasts

Equipment

2 microscope slides
1 cover slip
1 eye dropper

Materials

India-ink
ethanol

Time Requirements

30 minutes

Procedure

1. Thoroughly clean two microscope slides by wiping them with a lint-free towel or a tissue soaked in ethanol.
2. Place a small drop of water on the microscope slide. The drop must spread out, otherwise further measures are necessary to remove grease from the slide (see figure 6).
3. Use a glass rod to mix a drop of India ink with the evenly spread drop of water.
4. Place a cover slip at a 45° angle on the microscope slide in such a manner that the solution is collected in the space between the slide and slip and held by the properties of adhesion and cohesion (see figure 7).
5. Push the cover slip evenly across the entire surface of the microscope slide. The suspension is thus spread across the slide. The thickness of the film of liquid decreases (see figure 8).
6. Allow the smear to air dry.
7. Prepare a second smear, using a drop of bacterial or yeast suspension instead of a drop of water.
8. Compare both smears are then compared under a microscope set at 400X magnification. Open the condenser completely and use the brightest possible light source.

Explanations and Suggestions

Steps 1-7. Remarks for these steps correspond to those in the previous investigation. It is advisable to refer to that section where necessary.

Step 8. Students who observe bacteria with a microscope for the first time should definitely prepare a control slide for comparison, because beginners often have difficulty interpreting microscopic images.

Investigation E. The Safe Microscopy of Mold Using the Petri-Slide Technique (after E.R. Lucius & M. Fries, 1990)

Petri slides are extremely flat, disposable petri dishes that can be sealed tightly (height = 6 mm, inner diameter = 47 mm, area of the base plate = 52 x 75 mm). They are designed for counting microorganisms. Unknown microorganisms are placed on nutrient cardboard discs or membrane filters, and colonies are cultivated inside the chamber. The construction of the chambers also enables both the safe microscopy of sealed fungal cultures and the microscopy of sporangia from the side. A packet of petri slides containing 100 slides can be ordered from laboratory suppliers for $60 (DM 117) or ordered direct from the firm under the catalogue No. PD15 047 00 at the following address: Millipore GmbH, Hauptstraße 71-79, D-6236 Eschborn, FR Germany, Tel. 0049-619-64940. In suitable conditions, petri-slide cultures can be kept for several weeks, so 100 slides may be sufficient for several years.

Basal agar medium is suitable for the cultivation of various molds. Fungi do, however, also grow on other nutrients such as glucose nutrient agar, but in such cases they do grow more slowly.

Prerequisite Biological Knowledge

ability to use a microscope
knowledge of structure and reproduction of molds

Equipment

1 autoclave or pressure cooker
2 petri slides

1 bunsen burner
1 pasteur pipette, sterile
1 conical flask, 300 ml, wide necked
1 inoculation needle
household aluminum foil

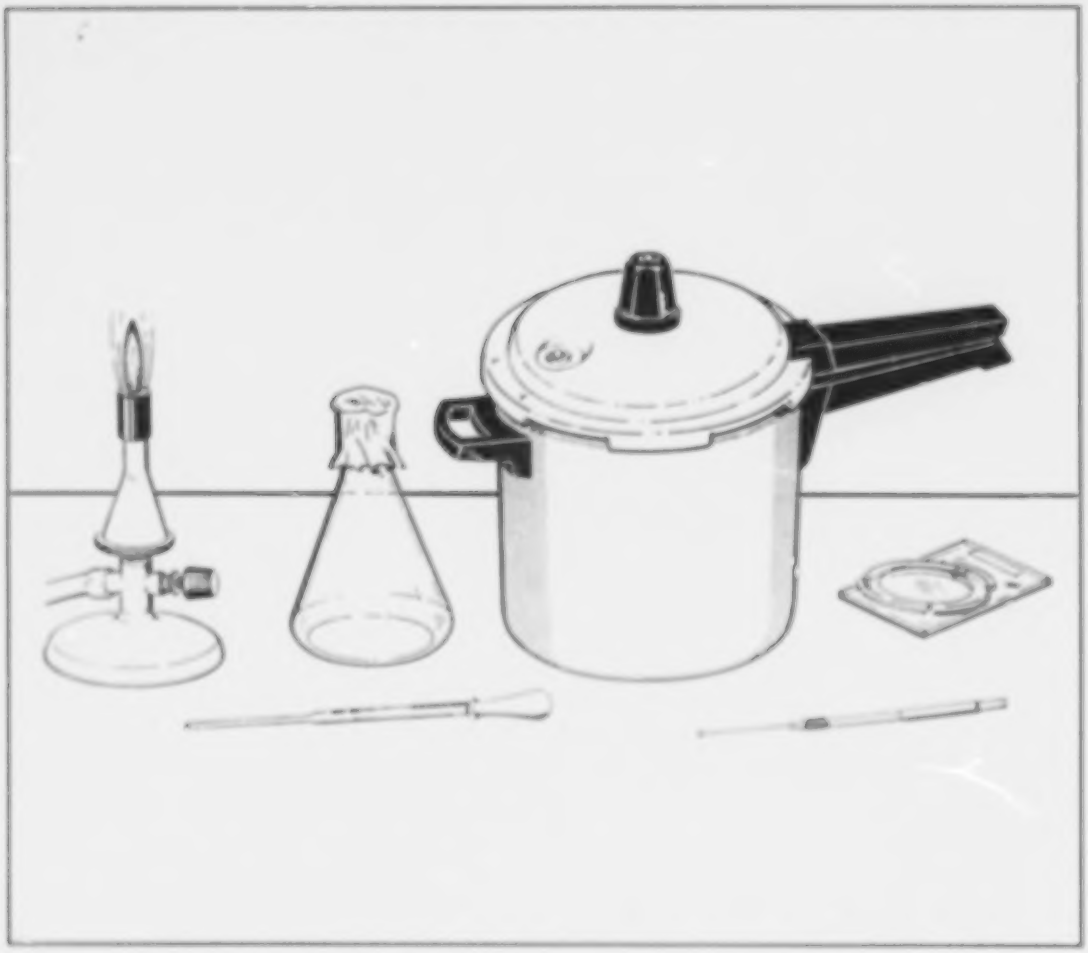

Figure 10. Required equipment.

Materials

basal agar medium, 200 ml (see appendix)
or glucose nutrient agar, 200 ml (see appendix)
1 n HCl
1 pure culture of *Penicillium camemberti* (DSM-No. 1995)
1 pure culture of *Mucor mucedo* (DSM-No. 809, ATCC 38693)

Time Requirements

45 minutes
inoculation of the petri slides: 15 minutes

Procedure

1. Place the culture medium in a conical flask, seal with aluminum foil, and autoclave in a pressure cooker. The time required for sterilization is 20 minutes after the sealing of the pressure valve.
2. Remove the aluminum foil from the conical flask. Fill a sterile pasteur pipette with 5 ml of sterilized culture medium. Do not touch the tip of the pipette. Hold the petri-slide chamber upright between the thumb and index finger of the left hand. Lift the lid up far enough to reveal the side of the base of the filling hole. Introduce the tip of the pipette through the hole in the side into the middle of the chamber. The tip must not touch the outer parts of the chamber. Carefully pipette approximately 3 ml of culture medium into the vertically held chamber without smearing the upper part of the chamber with culture medium.
3. Remove the pasteur pipette from the chamber and replace the lid of the chamber. Ensure that the chamber remains vertical as the agar sets. Refill the pipette and prepare additional chambers in a similar manner. Pass the tip of the pipette through a bunsen flame periodically.
4. After the agar has set inside the chambers for about 30 minutes, inoculate the chambers with different fungi. Use an inoculation needle that is sterilized by heating in the bunsen burner flame and held to the sporangia of a pure culture of mold.
5. Open a petri slide in the usual way. Insert the needle through the hole until the pointed end transfers the spores that adhere to it by contact with the surface of the agar.

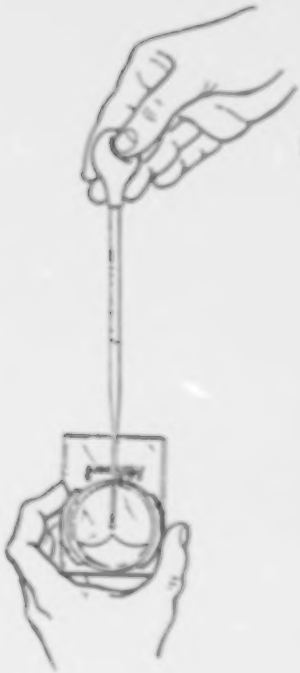

Figure 11. Filling a petri slide with culture medium.

Figure 12. Removing fungal spores from a pure culture.

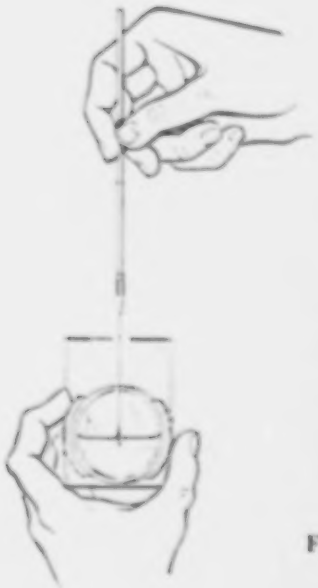

Figure 13. Inoculating the culture medium with fungal spores.

6. Close the chamber again and incubate it for about a week in a vertical position at room temperature.
7. Place the petri slides flat onto the stage of the microscope once the cultures have grown; examine with one of the two low-power objectives (10x or 40x).

Explanations and Suggestions

Step 1. The petri slides do not have to be sterilized, as they remain germ free inside during the production process. Sterilize pasteur pipettes by wrapping them in aluminum foil and heating them to 180°C for 30 minutes in a drying cabinet.

Step 6. Individual sporangia can be seen clearly even with microscopy using transmitted light. One can "take an optical walk" through the approximately 5 mm-wide sporangia "wood" using the fine-focusing apparatus. Differences in the sporangia at the edge of the colony and in the middle can be seen clearly, and the mass of hypha in the nutrient agar can be examined up to its finest traces. The petri-slide cultures can be kept for several weeks in a dark cabinet at room temperature. It is inadvisable to keep them in a refrigerator, as the cold chambers become slightly steamed up if they are used again. The cultures hardly dry out at room temperature, and the fungi cease growing one or two weeks after they are inoculated because they lack oxygen and nutrients.

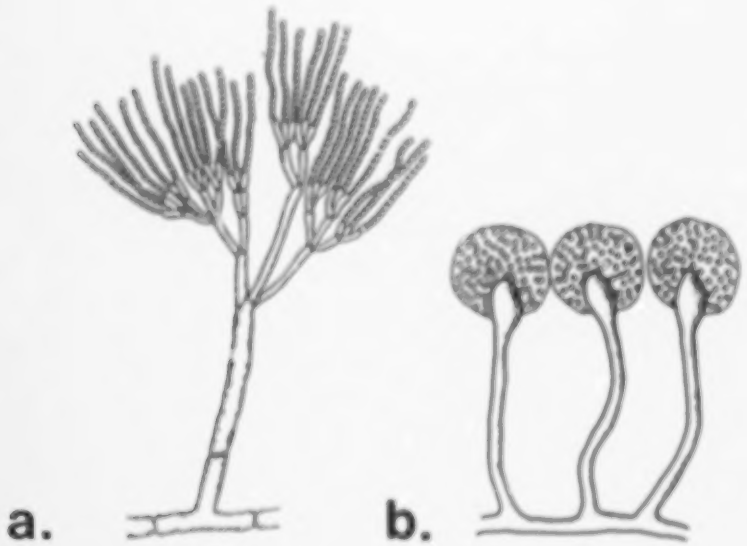

Figure 14. Side view of various molds under the microscope: a. *Penicillium*; b. *Mucor*.

Problems and Questions

1. Why should moldy fruit or bread not be examined using open microscopy? (The types of mold that grow on them are often of the genera *Penicillium* and *Aspergillus*; the spores of the latter may be harmful if people inhale them; *Aspergillus flavus* also produces a poison--aflatoxin.)
2. Why is it impossible to determine the genus of a fungal colony, and why is it impossible to compare various species of mold by microscopic examination from above? (The sporangia, which may be used to distinguish one genus from another, can only be seen from the side.)
3. Why must the tip of the pipette not touch the outside of the petri slide when the slide is being filled with agar? (In this case, the pipette is contaminated with bacteria or fungal spores from the environment; the spores soon begin to sprout and grow in the sterilized agar. Any additional experiment with a nutrient base that has been contaminated in this way is invalid.)

3. *Screening: Identifying Microorganisms that Produce Certain Effects.*

Scientists from the Bayer company in Leverkusen transferred water samples from the sections of the Rhine that were most heavily polluted with industrial effluent onto culture media that contained a dioxin compound (Seveso poison) as its main source of carbohydrates. They hoped to find bacteria that could decompose this dangerous industrial waste product. Colonies of bacteria actually did grow. Surprisingly, these were brevibacteria, which can usually decompose lactose and are found on cows' udders and in dairy products. The ability to decompose dioxin results from spontaneous mutation. The mutants and their progeny found sufficient nutrients in the effluent to keep them alive.

Various microorganisms can perform innumerable services during the construction and decomposition of substances. Industry uses bacteria or fungi and attempts to discover through screening procedures microorganisms that might help to solve a certain problem. The prospects of success are very good. One might even say that there is no problem in this area that a certain microorganism cannot solve, regardless of whether the problem relates to the decomposition of crude oil or the waste products of the chemical industry, the synthesis of dyes, the production of biopolymers, or the production of antibiotics. For these purposes, one looks for microorganisms that occur naturally. One expects, for example, to find crude-oil catabolites in the vicinity of sources of crude oil, and one examines laboratory strains that one has already subjected to mutagenic agents for mutants that can be used. The following section includes a screening procedure designed to trace soil bacteria that decompose urea. In addition, there are investigations that demonstrate the production and effect of an antibiotic and that explore the antibiotic effect of other substances.

Investigation F. **Tracing Soil Bacteria that Decompose Urea (by E.R. Lucius, after R. Westphal, 1989)**

Urea is the product of the decomposition of protein, for example, in the human body and among mammalian livestock. It is released into the environment in large quantities in urine. Certain bacteria can decompose urea, which they use as a source of food. In the following experiment, bacteria are isolated from soil. Initially, they are given a rich culture medium on which almost all of the bacteria form colonies. In a parallel investigation, an extremely "selective" soil is used. This contains urea as a principal source of nutrition, and practically only those bacteria that are able to form the enzyme urease, which decomposes urea, are able to grow on it.

We are concerned with a model experiment for industrial screening. The bacterial suspension that is isolated from soil must be diluted, as discrete colonies only grow on the culture medium when there is a low concentration of bacteria. There are, after all, up to 10^9 viable microorganisms in a gram of soil.

Prerequisite Biological Knowledge

the ecological significance of soil bacteria as decomposers
the enzymatic decomposition of urea

Equipment

Drigalski spatula
incubator
10 basal agar medium plates (see appendix)
5 urea agar plates (see appendix)
1 beaker, 400 ml
2 bunsen burners
1 graduated cylinder, 10 ml
1 eye dropper, sterile
1 glass plate
6 test tubes, sterile
1 waterproof felt-tip marking pen

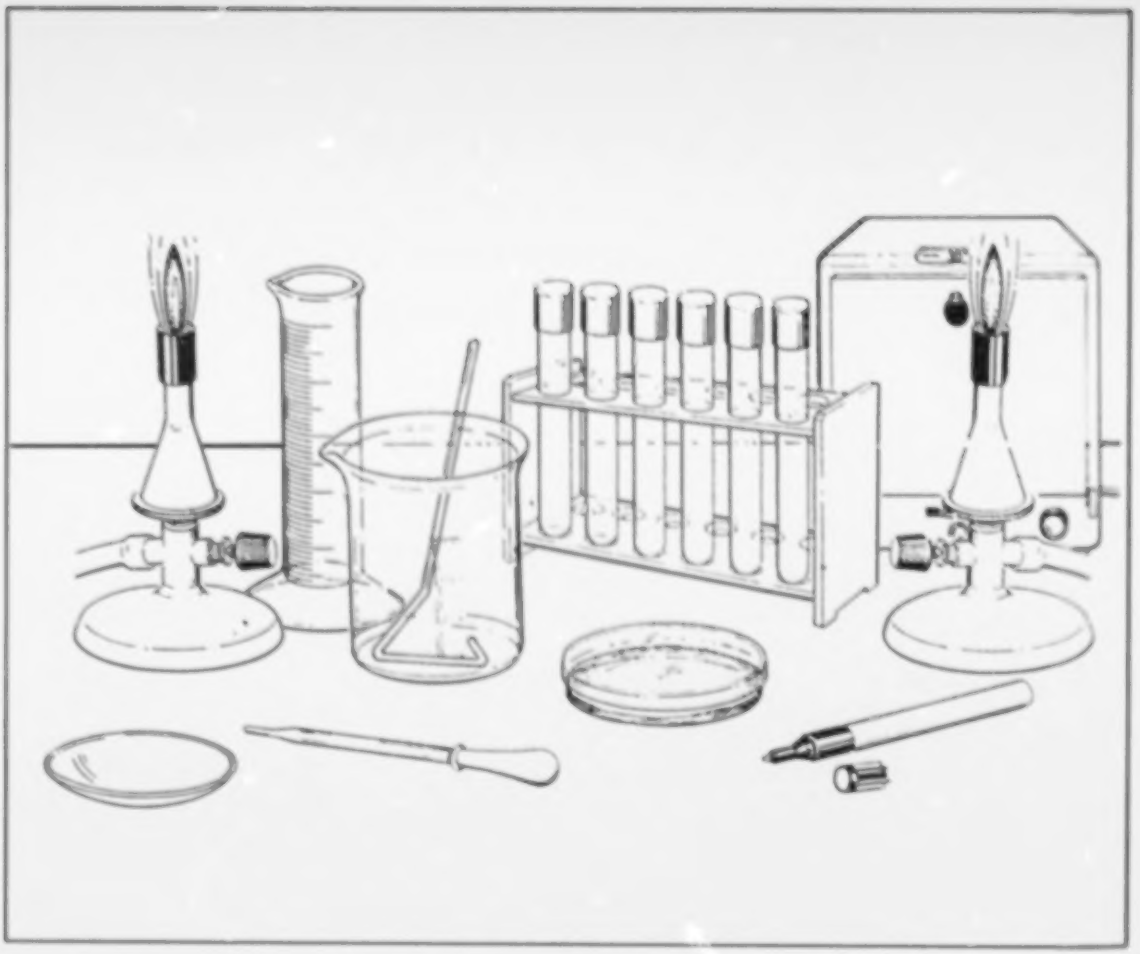

Figure 15. Required equipment.

Materials

1 g soil
ringer solution, sterile, 500 ml (see appendix) or salt solution (see appendix) to dilute the microorganism suspension

Time Requirements

steps 1 - 8: 45 minutes

Procedure

1. Label six sterile test tubes in series as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.
2. Label the undersides of the agar dishes in series as follows: Nutrient plates: K, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}; urea agar plates: K, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}.
3. Fill the beaker with roughly 50 ml ethanol and light the bunsen burners.
4. Conduct the following steps between the two burners. DANGER: Ethanol is highly flammable. If it ignites, extinguish it by covering the container with a glass plate.
5. Pipette 10 ml of the Ringer solution into the test tube labelled 10^{-1}.
6. Sterilize the Drigalski spatula by flame heating. Remove the spatula from the ethanol filled beaker in which it was placed at the beginning of the experiment; allow it to drip thoroughly. Burn off any remaining alcohol by rotating the spatula in the flame. Be careful to prevent burning alcohol from dripping. Hold the spatula downwards to avoid the danger of burned fingers from burning alcohol trickling down the handle. Apply a drop of sterile Ringer solution to the plate labelled "K"; spread the solution evenly over the surface of the dish with the sterilized spatula (figure 16).
7. Add one gram of soil to the test tube labelled 10^{-1}. Mix thoroughly by shaking and by rolling the test tube back and forth between the palm of the hands. Apply one drop of the 10^{-1} suspension to each of the two plates labelled 10^{-1}; spread the suspension evenly with the re-sterilized spatula (according to step 5). Finally, rinse the pipette with sterile water.
8. Transfer one drop from the test tube labelled 10^{-1} to the test tube labelled 10^{-2}; mix carefully again. Distribute one drop of the 10^{-2} suspension evenly on each of the two petri dishes labelled 10^{-2}.
9. Proceed in the same manner until all other test tubes are filled, each with one drop of suspension from the previous tube and nine drops of water, and until all petri dishes are inoculated according to their respective labels.
10. Incubate the dishes for seven days at 30°C.
11. Count the colonies as soon as they can be easily recognized. Make complete counts of plates with 50 to 200 colonies.
12. To determine the titer of the suspension, one must determine the volume of one drop. For this purpose, fill a small graduated cylinder with drops (n) to a volume

of 2 ml. The volume (V) of a drop is derived according to the following formula:

$$V = \frac{2}{n} \text{ ml}$$

Example: 32 drops are necessary to attain a volume of 2 ml. The volume of a drop is therefore:

$$V = \frac{2}{32} \text{ ml} = 0.06 \text{ ml}$$

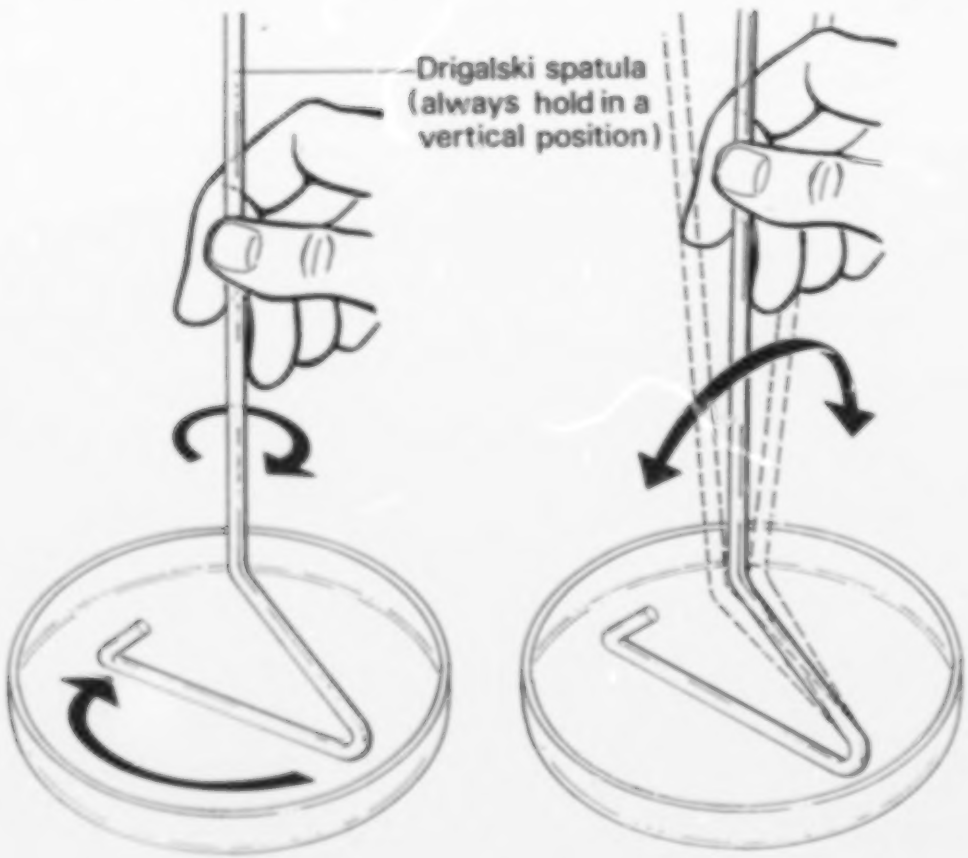

Figure 16. Proper use of Drigalski spatula.

13. If a set of identical eye droppers is available, it is sufficient to allow a student who works quickly to determine the drop volume in order to save time. The other groups can use these results. Divide the number of counted colonies by the dilution factor. The results indicate the number of viable cells in one drop of the suspension. When this number is subsequently divided by the drop volume (V), the number of viable cells in 1 ml of the suspension is obtained.

Example: 186 colonies were counted on the plate labelled 10^{-4}. The volume of a drop is 0.06 ml. The number of colonies is divided by the dilution factor:

$$N = \frac{186 \text{ cells}}{10^{-4}}$$

Divide by the volume (V) of drops:

$$N = \frac{1.86 \times 10^{6} \text{ cells}}{0.06 \text{ ml}} = 3.1 \times 10^{7} \text{ cells per ml.}$$

Explanations and Suggestions

Steps 1 and 2. Inadequate labelling is a common cause of mistakes, especially when working with young students. Therefore, teachers must always insist on careful and adequate labelling. Avoid simplified symbols such A, B, C. This method requires a deciphering key, the production and use of which also can lead to careless mistakes. Always write labels on the underside of the plate, because lids can be exchanged by mistake.

Step 3. The risks of contamination during this phase are immense; we strongly recommend that the students conduct procedures of pipetting and inoculating between two burners. The burners produce a steady current of rising heat, which prevents microbes in the air from forming sediment and helps to keep them from precipitating into culture mediums and containers (figure 17). Open flames in the laboratory, however, require the students to take great care. The teacher must decide from case to case what the students can safely handle.

Step 5. It is important to begin with the control sample because the sterile water is subsequently needed for rinsing and consequently becomes contaminated.

Step 6. As with yeast, bacteria are transferred in conglomerates. They must be separated from each other because they will otherwise not disperse evenly in the suspension. The mixing may well be the main cause of error in this experiment, as students often

underestimate the importance of the mixing process and the separation of the lumps and their inherent practical difficulties. It is advisable to practice the mixing process beforehand with 0.5 ml stain and 45 ml water.

Step 10. If colonies of less than 50 are counted, the effects of random variation are too great. When there are more than 200 colonies, counting is complicated because numerous colonies tend to grow into one another. .

Step 11. This procedure is possible because the volume of a drop in a pipette generally remains constant, provided the viscosity of the liquid used remains constant.

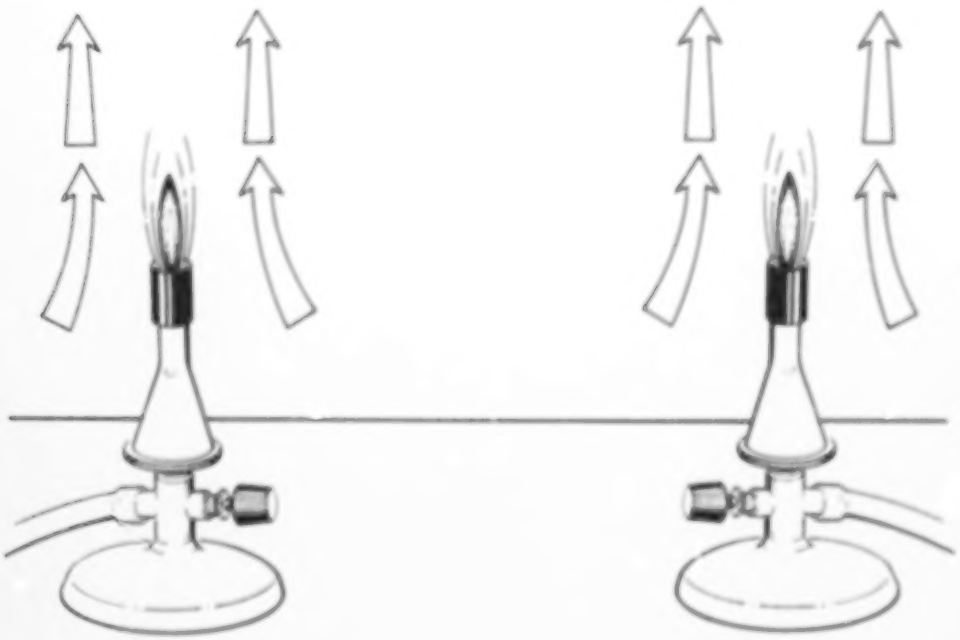

Figure 17. Ascending air current near the burner.

Problems and Questions

1. Why are bacterial colonies on plates that have been diluted very little (10^3, 10^4) smaller than colonies on plates that have been diluted a great deal (10^6, 10^7)? (The bacterial colonies compete for the nutrients in the agar; on plates where the concentration is high, more colonies develop but they are more prone to "starve" than few colonies on plates that are very dilute.)

2. Why do fewer colonies grow on urea agar plates than on basal medium plates at a comparable concentration? (Only very few microorganisms possess urease and can use urea as a source of carbohydrates for biosynthesis. Most can, however, use glucose, which is present in large quantities in basal medium.)

3. The decomposition of urea is visible by means of a red indicator zone. What substance produces this proof? (During the decomposition of urea, a basic ammonia is excreted from the bacterial cells and released to the environment.)

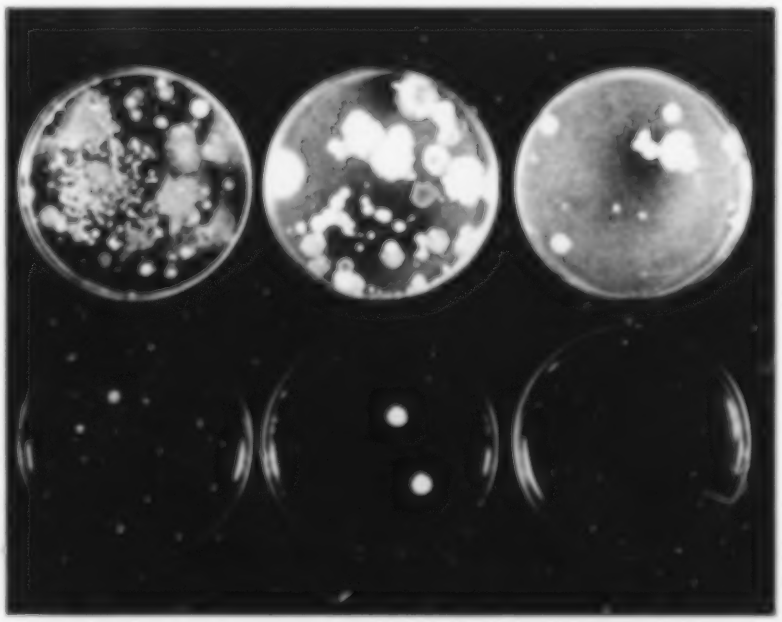

Figure 18. Agar plates of dilution series (10^3, 10^4, 10^5) of soil, a. basal agar medium, b. urea agar medium.

Investigation G. **Demonstrating Production of an Antibiotic (by E.R. Lucius & M. Fries)**

The phenomenon of bacterial resistance keeps humans dependent on the development of new antibiotics. These are produced by the chemical alteration of antibiotics already in existence, or by tracing microorganism that produce antibiotics that were heretofore unknown. Producers of antibiotics naturally occur fairly frequently. By producing these substances, they keep possible competitors "at bay." They also occur regularly in the screening process described in the previous investigation.

The following investigation employs *Streptomyces griseus*, whose ability to produce the antibiotic streptomycin is well known. Only those test organisms that are not sensitive to streptomycin can grow on the same culture medium plate as *Streptomyces griseus*. The use of *Streptomyces* or streptomycin is possible in school experiments because this antibiotic is no longer used in medicine, and the possible spread of resistent strains is no longer problematic from a medical point of view.

Prerequisite Biological Knowledge

origin and effects of antibiotics
the development of resistance

Equipment

1 autoclave
1 incubator
1 bunsen burner
8 disposable petri dishes
2 sterile, 300 ml conical flasks with sterile bungs
1 inoculating loop
6 culture tubes
5 x 5 ml sterile pipettes
pipette aid

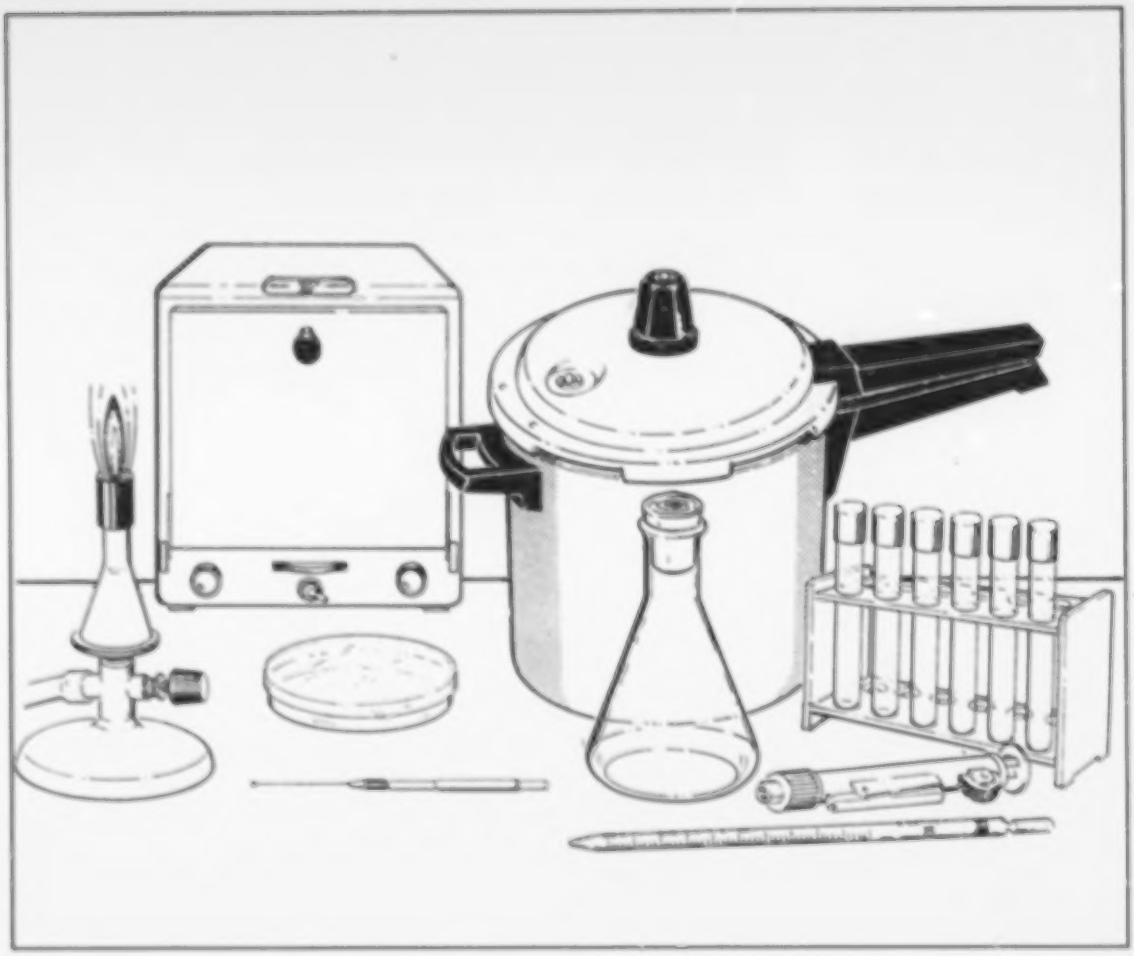

Figure 19. Required equipment.

Materials

pure culture of *Streptomyces griseus* (DSM-NO. 40236, ATCC 23345)
a selection of the following pure culture strains as test organisms:
Bacillus mycoides (DSM-No. I0, ATCC 6051)
Candida utilis (DSM-No. 2361, ATCC 9950)
Eschericia coli K-12 (DSM-No. 498, ATCC 23716)
Micrococcus luteus (DSM-No. 20030, ATCC 4698)
Pseudomonas fluorescens (DSM-No. 50090, ATCC 13525)
distilled water

basal broth medium for overnight cultures, 100 ml (see appendix)
basal agar medium for petri dishes, 200 ml (see appendix)

Time Requirements

preparation and autoclaving of the solutions: 45 minutes
preparation of the precultures: 15 minutes
waiting time: 24 hours
distribution of the test organism cultures: 16 minutes

Preparation

Prepare and autoclave the culture media. Place the basal agar medium into eight petri dishes (approximately 20 to 25 ml per plate). Re-suspend the culture of *Streptomyces griseus* in 1 ml of sterile basal broth medium according to the manufacturer's instructions; pipette into a test tube into which 5 ml sterile culture medium has been placed. Incubate for 24 hours in an incubator at 30°C (overnight culture).

Procedure

1. Inoculate the basal agar media in the petri dishes with the overnight culture of *Streptomyces griseus*. Sterilize an inoculation loop by passing it through a bunsen burner flame; allow the loop to cool, then dip into the culture. Streak the germs that adhere to the loop onto the culture medium as a vertical line as far over to the right as possible so that the left part of the surface of the culture medium remains sterile. Sterilize the inoculation loop by passing it through a flame once more.
2. Incubate the plates for two days at 30°C.
3. In the interim (after incubating for 24 hours), prepare the overnight cultures of the selected organism (*Bacillus mycoides, Candida utilis, Escherichia coli* K-12, *Micrococcus luteus, Pseudomonas fluorescens*) using the same procedure as for the overnight culture of *Streptomyces griseus*.
4. Once the incubation time has elapsed, inoculate the culture media from four petri dishes on which the *Streptomyces* cultures are now visibly growing with the test organism; use an inoculation loop (as in step 1) to make horizontal streaks on the empty part of the medium (the left side of the dish). The streaks must always be vertical to the *Streptomyces* culture and be drawn close up to the edge of it, but it

must not be touched or contamination will occur. Keep cultures overnight.

5. Incubate the plates for two days at 30°C and keep them in a cool place for a day longer.
6. In the interim (after incubating for 24 hours), inoculate the culture media of the other four petri dishes, on which the *Streptomyces* cultures are now even more vigorous, with the test organisms using an inoculation loop as in step 4.
7. Incubate the pates for two days at 30°C.
8. Compare and assess the first and second set of four plates.

Problems and Questions

1. Why do some test organisms grow in the vicinity of the *Streptomyces* culture and others not? (*Streptomyces griseus* produces an antibiotic--streptomycin--that diffuses into the culture medium. Some organisms (*Bacillus mycoides, E. coli, Micrococcus luteus*) are sensitive, that is, they are killed off by the antibiotic at a certain concentration; others are resistant. Yeasts (*Candida utilis*) are not susceptible to streptomycin because they are eucaryotes.)
2. Why is the distance between sensitive test organisms and the *Streptomyces* culture larger in older streptomyces cultures? (Production of antibiotic increases in the older cultures.)

Investigation II. Demonstrating the Effect of Streptomycin in the Small-Disc Test (by E.R. Lucius & M. Fries, after R. Westphal, 1989)

Many species of fungus release antibiotics into the environment. These compounds have a bacteriostatic effect (which inhibits growth and reproduction) or a bactericidal effect (which kills bacteria) that serve as a means of competition between species. In 1928, Flemming discovered that it was possible to use these compounds in medicine to combat infections caused by bacteria in humans. Penicillin has been produced on an industrial scale since 1940; since then many other antibiotics have been discovered. Their effectiveness can be tested by using the small-disc test (after H. Schrempf, 1985).

Prerequisite Biological Knowledge

origin and effects of antibiotics
the formation of resistance
mutation

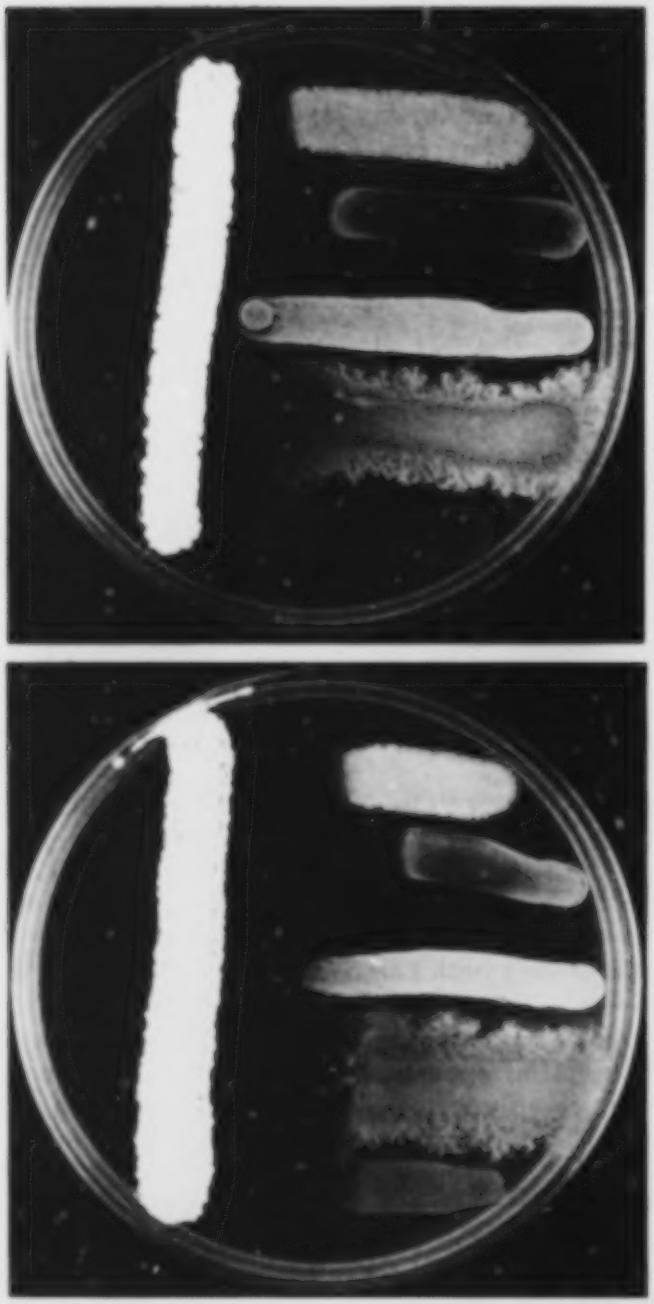

Figure 20. Comparing antibiotic streak tests. Two- and three-day-old streptomyces cultures. Test organisms from above: *Candida utilis, Micrococcus luteus, Pseudomonas fluorescens, Bacillus mycoides, Escherichia coli.*

Equipment

petri dishes
1 paper punch, or pair of scissors
1 pair of tweezers
1 bunsen burner
1 small glass beaker
1 large glass beaker as a water bath
needles
corks and glass beaker or test tubes with rubber bungs
filter paper
aluminum foil

Materials

pure culture of *Bacillus subtilis* (DSM-No. 10, ATCC 6051)
a yeast suspension that was incubated overnight (1 g yeast to 100 ml water) nutrient agar medium, 200 ml (see appendix)
malt agar medium, 200 ml (see appendix)
streptomycin or another antibiotic
ethanol

Procedure

1. Prepare nutrient agar as a bacterial culture medium and malt agar as a yeast culture medium.
2. Sterilize the culture media and cool them in a water bath to 40°C; keep them at this temperature.
3. Add 1 ml of a culture of *B. subtilis* or yeast suspension (which was incubated overnight) per 200 ml to the still-liquid culture medium; close the conical flask and mix the contents vigorously.
4. Pour the culture medium that is inoculated in this way into petri dishes and allow it to set.
5. Use a hole punch or a pair of scissors to produce small discs (0 = 5 mm) from a sheet of filter paper.
6. Sterilize two small discs by placing each one on a needle and then into ethanol (96%) overnight or by sticking them into a cork and placing them in a glass beaker

sealed with aluminum foil and then into a drying cabinet at 135°C for three hours. Exposure to ultraviolet light for ten minutes will also work.

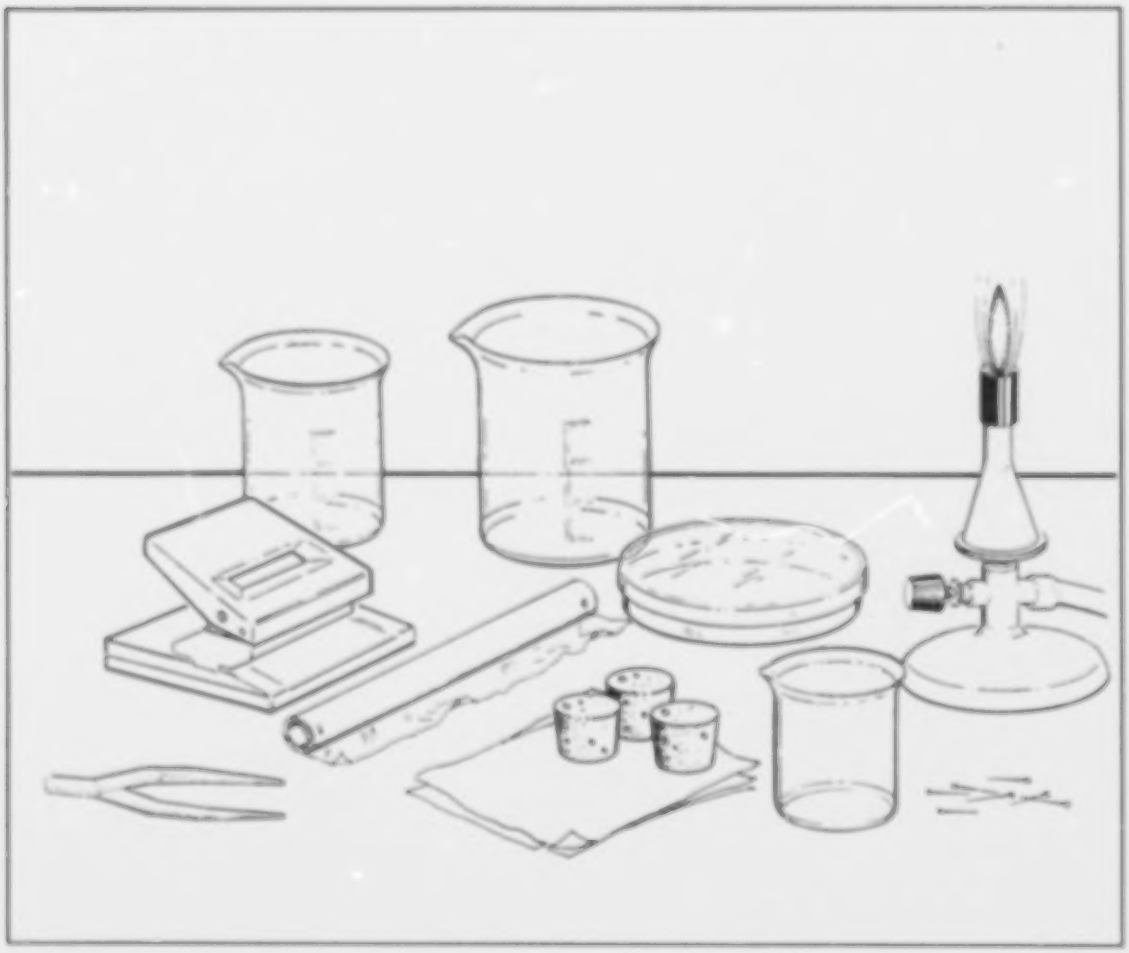

Figure 21. Required equipment.

7. Produce a solution of streptomycin in sterile water. The concentration of the pure agent should be about 50 mg/ml.
8. Dip the sterile small discs into the antibiotic solution and dry them in a drying cabinet at 100°C. Dip at least one small disc into sterile water free of antibiotic, as a control.
9. Use a sterilized pair of tweezers to place each of the dried antibiotic plates onto an inoculated labelled agar plate. As a control, use at least one petri dish without a small disc.

10. Incubate the closed petri dishes at 30°C for two days. A uniform bacterial or yeast lawn should be present on all of the control plates, and a circular zone free of bacteria should be visible around the small discs that contained antibiotics. The yeast is not affected by the antibiotics.

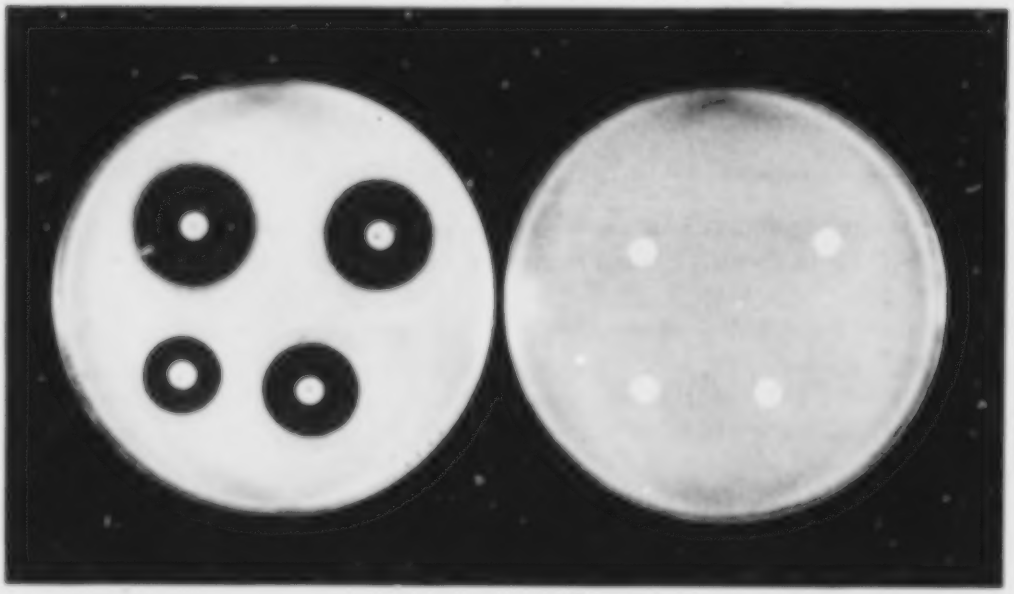

Figure 22. Comparing the streptomycin sensitivity of microbial strains, a. *Bacillus subtilis* b. *Saccharomyces cerevisiae*.

Explanations and Suggestions

Steps 2 and 3. Cooling is necessary because the hot agar would damage the organisms. If the agar is left to cool without putting it into a water bath, it sets too quickly.

Step 8. If the damp paper discs were placed onto the agar, the agents would begin to diffuse uncontrollably.

Step 9. The addition of small paper control discs shows that filter paper itself does not contain any substances that inhibit the growth of bacteria. The control plate without the small paper discs shows that a completely uniform bacterial lawn develops on an untreated plate.

Step 10. The antibiotic gradually diffuses into the agar. The diameter of the zone in which no bacteria grow is a measure of the concentration of the agent. If two different biocatalyst solutions of a similar concentration were used, the diameter of the bacteria-free zone would be a measure of the toxicity to the bacteria used.

Problems and Questions

1. What is the significance of colonies that grow within an antibiotic bacteria-free zone? (Each of them is formed from one mutant of the bacterium that is resistant to the antibiotic.)
2. Can these resistant microorganisms damage one's health? (No. The microorganisms do not pose a danger for people. Streptomycin is no longer used in medicine. Even a transfer of resistance to other microorganisms would not, therefore, have any effect on human health.)

Investigation I. **Demonstrating the Presence of Bactericidal Substances (by E.R. Lucius & M. Fries)**

If a coin is placed onto a culture medium that is uniformly inoculated with *Bacillus mycoides*, a bacterial lawn grows with a bacterial free zone around the coin. The coin may consist of German silver, an alloy of copper, nickel, and zinc. Their metal anions kill cells of *B. mycoides* by inhibiting growth and division. From the side, it is obvious that the colony is more dense at the edge of the zone than in the rest of the bacterial lawn. The metal anions encourage growth in small quantities.

An area of resistant microorganisms is often formed in the immediate vicinity of the coin. These microorganisms can be traced to the coin and have become enriched in the course of time. They are resistant to ions of heavy metals. The demonstration is therefore also indicative of the pressure of selection that bactericides exert on a population of microorganisms. This problem occurs quite frequently in hospitals, where certain microorganisms suddenly occur in large numbers, for example, the bacterium *Serratia marcescens*.

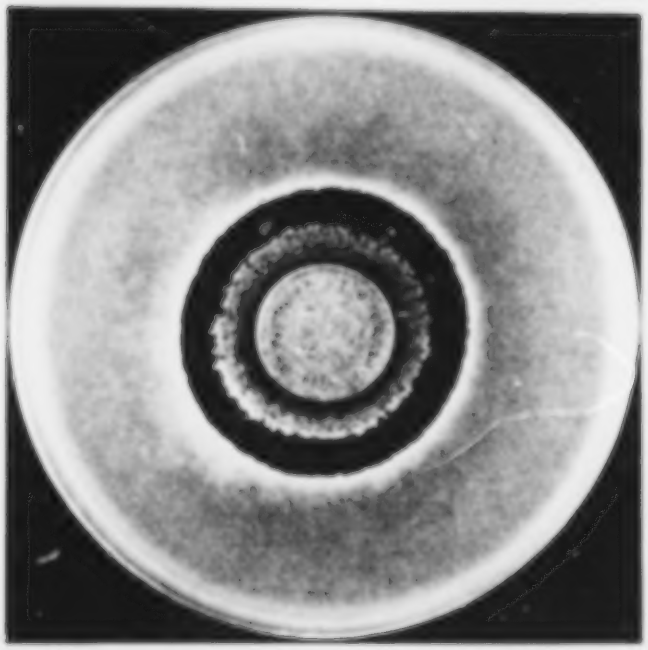

Figure 23. Inhibiting and encouraging growth of microorganisms by the use of bactericidal substances.

Prerequisite Biological Knowledge

the formation of resistance
mutation
selection

Equipment

1 autoclave
1 incubator
1 bunsen burner
4 disposable petri dishes
1 300 ml sterile conical flask
bung
6 culture tubes

adhesive tape for sealing the petri dishes
5 x 5 ml sterile pipettes

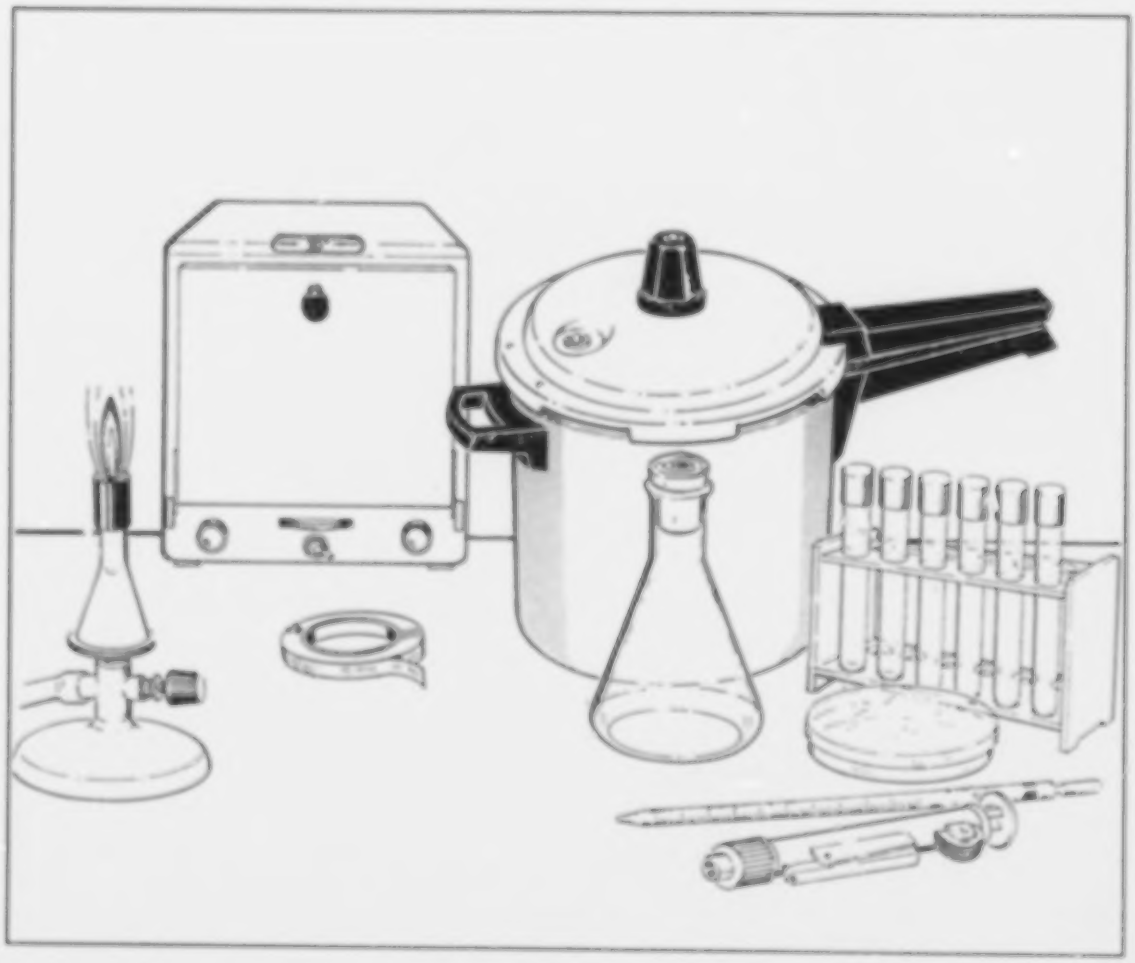

Figure 24. Required equipment.

Materials

pure culture of *Bacillus mycoides* (DSM-No. 2048, ATCC 6462)
distilled water
100 ml basal broth medium for cultures incubated overnight (see appendix)
100 ml basal agar medium (see appendix)

Time Requirements

preparation and autoclaving of the nutrient solution: 45 minutes
preparation of the overnight culture: 15 minutes; waiting time: 48 hours

Preparation

Prepare and autoclave the basal broth medium. Re-suspend the culture of *Bacillus mycoides* in 1 ml of sterile liquid basal medium according to the manufacturer's instruction; pipette this into a test tube previously filled with 5 ml of the sterile liquid basal medium. Incubate for 24 hours at 30°C (culture incubated overnight).

Procedure

1. Place the basal agar medium into conical flasks; autoclave and cool to approximately 45°C under running water. The approximate temperature has been reached if you can hold the warm conical flasks to the back of your hand with no unpleasant sensation (back-of-hand test).
2. Add the culture that was incubated overnight and mix well with the culture medium by swirling the contents of the flask.
3. Pour the inoculated culture medium into four petri dishes.
4. Once the agar is set, place a coin onto the surface of the culture medium in the middle of the petri dish.
5. Close the petri dishes and seal them with adhesive tape.

Problems and Questions

1. Why must the petri dishes be protected against accidental opening, and why must they therefore be sealed? (Microorganisms that may grow on the coin, and possibly on the culture medium, are unknown; any risk that wild strains may pose are avoided in this way.)

Fermentation Systems

"Where do bacteria live? Have they got a home?" Teachers who produce yogurt or sauerkraut with nine-year-old-students should be prepared to answer such questions. These questions may seem burdensome, but the teachers will be compensated by the enthusiasm and curiosity of the students who are given the opportunity to explore traditional biological processes used in the manufacture of food. Before the lessons, if students were asked where yogurt and sauerkraut come from, the only answer that many could give is "from the supermarket." These lessons will help the students understand the process of fermentation properly for the first time and will introduce them to some extremely old culture techniques that were certainly part of family life before they became the province of large, specialized firms not so long ago.

Older students can learn about fermentation in these areas and discuss the technical and ecological problems of mass food production. The combined production of alcohol and vinegar introduces questions of design and engineering, scaling up and downstream processing, and the disposal of large quantities of biomass. Fermentation also occurs in the decomposition of organic waste (for example, in sewage works), in the production of compost, or in other natural processes. These processes can be represented in a simple model experiment by placing paper onto damp soil from a wood; the paper gradually rots. During this process, soil microorganisms produce the enzyme cellulase, which splits the cellulose of the cigarette paper into its glucose components, which are absorbed by the microorganisms and decomposed.

1. ***Fermentation Processes in Food Production.***

Investigation J. How are Yogurt and Sauerkraut Produced? A Teaching Unit on Biotechnology for Grade Four Students (after H. Bayrhuber, M. Fries, and Th. Heinen, 1990)

J1. Production of Yogurt

Prerequisite Biological Knowledge

No special knowledge is required.

Equipment

1 balloon whisk or one wooden spoon

20 cups or glasses
1 oven ring
1 saucepan
20 teaspoons
1 thermometer (100°C)
incubator or insulated box made out of polystyrene foam (dimensions: 20 cm high x 35 cm long x 30 cm wide; thickness of the polystyrene: 6 cm) or "yogurt machine"

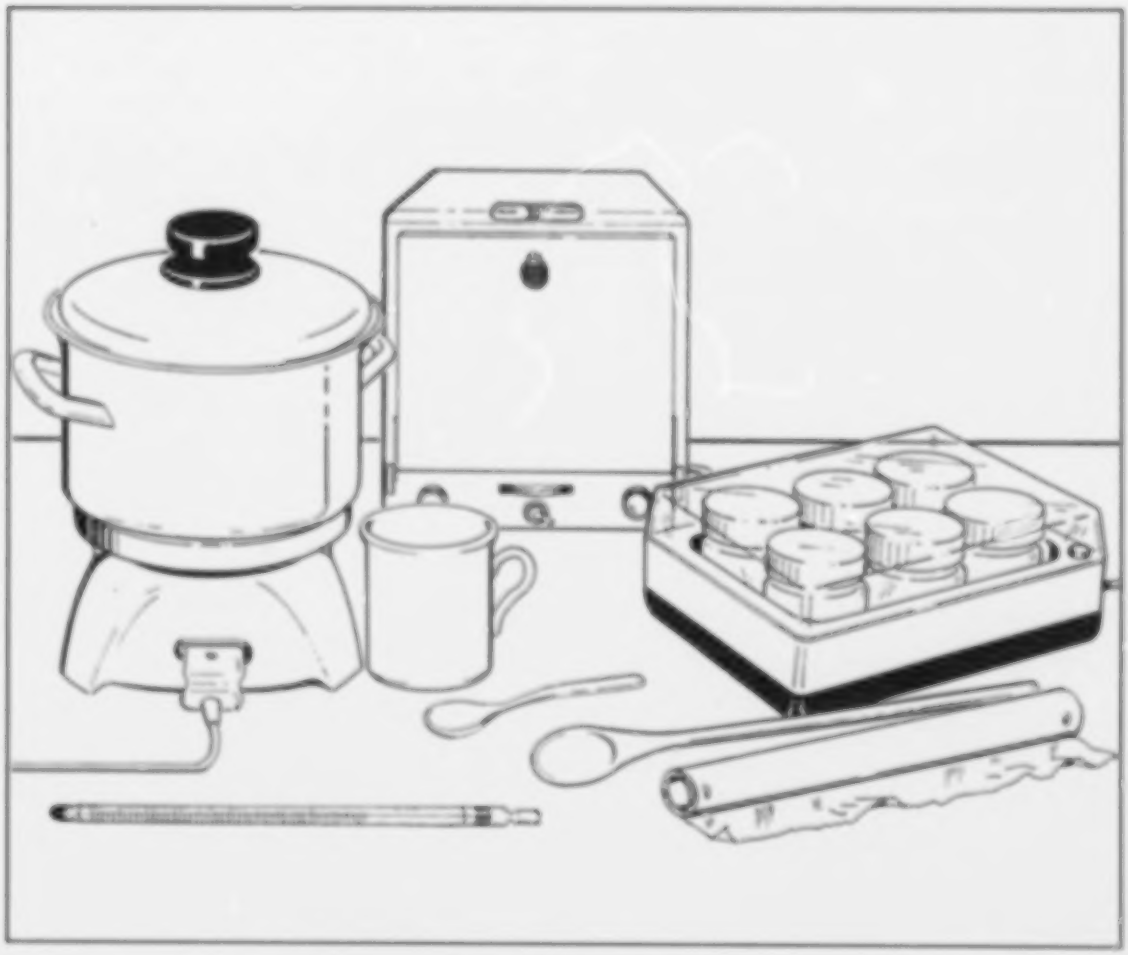

Figure 25. Required equipment.

Materials

3 liters of milk
3 beakers of yogurt made from whole milk
cling film

Procedure

1. Heat the milk to 72°C; this kills harmful bacteria in the milk.
2. Allow the milk to cool to 45°C.
3. Place a teaspoon of yogurt and lactic acid bacteria into a beaker.
4. Add the cooled milk to the beaker.
5. Mix all of the ingredients.
6. Cover the beaker with cling film.
7. Place the yogurt mixture into an insulated box.
8. After several hours the milk has thickened. The yogurt is ready; it tastes acidic.

a. Educational and methodical considerations

By producing yogurt and sauerkraut, nine-year-old-students become familiar with two traditional biotechnological procedures. They discover that in both cases bacteria ensure that the milk thickens and the sauerkraut is not spoiled. These concepts can be taught without overtaxing the students and without explaining the process by which lactic acid is produced or that the acid is a product of the fermentation of milk. Young children would likely not understand that lactic acid bacertia decompose sugar to lactic acid and release energy in the process. They can, however, understand that milk does not thicken of its own accord, that sauerkraut does not become sour of its own accord, and that both phenomena can be traced to the activity of very small organisms called bacteria. The students need not be told that lactic acid bacteria are at work in both cases. The term "lactic acid bacteria" is used only for the bacteria in yogurt, not for those in sauerkraut. The latter are referred to as "sauerkraut bacteria" by the students. It is reasonable to use the scientific term in the first case, because the bacteria actually make the milk sour. In the second case, this term is not used because we expect that the students will ask whether sauerkraut contains milk--an unnecessary digression.

Students at this age have often heard of bacteria, but may attribute to them characteristics of higher organisms, even of humans. Students are eager to ask questions about bacteria, and their questions therefore play an important role in

such teaching. Teachers should attempt to impart to the students a concept of bacteria that is appropriate for this stage in their development.

This lesson produces foods that the students know from everyday life, and they should be allowed to try what they have made. Taste and smell, therefore, also play an important role. The palates of the students differ, and not all students will find the smell of sour milk pleasant. Yet, unpleasant experiences do not ruin the student's enjoyment; even if something smells nasty, at least it smells nice and nasty. From the start, there is no guarantee that the students will not find yogurt and sauerkraut repugnant when they discover that these foods contained bacteria. This fear is not confirmed in practice, however. For example, the children we worked with differed in their reasons for not liking yogurt, and clearly expressed those reasons. Almost all of the students liked the sauerkraut, however.

b. Lesson plan--Making yogurt from milk
Day 1, Double Lesson

One day in advance of the lesson, ask the students to bring a beaker (a jar) and a spoon to school with them. The beginning of the lesson will be marked by a slight clatter, and by the expectation--evident in the students' faces--of something delicious to eat.

Teacher: "Today we are going to make yogurt. What does one need to make it?"
Students: "Fruit, sugar, cream." "Oh yes, milk as well."

The teacher explains that bacteria are also necessary. The children seem amazed. One even seems shocked: "I'm not going to eat any bacteria."

Teacher: "What do you know about bacteria?"

Students: "If you swallow them, you are ill." "If you are ill and cough at somebody they are ill as well." It is not surprising that the term "bacteria" has a negative connotation for children. They have been taught to treat bacteria with respect--even fear--up to now. One student may, however, know that "there are good and bad bacteria."

Help the class differentiate between "useful" and "harmful" bacteria. They know that bacteria are tiny and that they can be seen only with the help of a microscope. The children also learn from the teacher that bacteria occur everywhere and that the bacteria used in the production of yogurt are called "lactic acid bacteria" ("yogurt tastes acidic!"). They also hear that bacteria are neither little animals nor little plants, and that it is not dangerous to eat lactic acid bacteria in yogurt. This introduction should take about 25 minutes. The investigation begins

next, as the teacher writes the individual steps on the blackboard. Students may copy the steps into exercise books (see "procedure"), but this is not necessary.

Each student heats 100 ml of milk. It is advisable to split each liter into several portions and to heat each portion separately, otherwise cooling takes too long. Some children may already be able to explain the necessity of heating: "The yogurt can be kept longer." "Otherwise, it would go bad." Many may have an idea of how hot 72°C is. "You can put your finger in it, but you have to take it straight out again, otherwise you burn yourself." All may be annoyed by the fact that the milk has to be cooled: "That takes a long time!" "This is boring!"

After cooling, add the yogurt to the milk; 3 beakers of yogurt are required for one class.

In the next lesson, fill the beakers. After stirring the mixture, the students automatically lick their spoons, which must be washed carefully in hot water before use. The milk-yogurt mixture tastes horrible. Tell the students not to put the licked spoons into the mixture again, because that will introduce harmful bacteria in the mixture.

The teacher now explains the function of the insulated box: "The foam walls only let a small amount of heat escape permanently. The milk stays warm for a long time. Lactic acid bacteria make yogurt fastest from milk at 42°C." Use the rest of the time to review information about bacteria. The most important points are as follows: Bacteria are tiny organisms that we cannot see with the naked eye. Some can cause illnesses and are therefore harmful. Others are very useful, for example, in making yogurt and sauerkraut. Most bacteria are neither useful nor harmful. The lesson continues on the following day: lactic acid bacteria make acid out of some milk. The acid thickens the rest of the milk and gives yogurt its acidic taste.

For homework, ask the students to draw the equipment needed to make yogurt (figure 26) and to consider how many bacteria standing next to one another could fit onto the thin edge of a piece of paper. Ask the students to imagine that the bacteria "are holding each other's hands" and forming a chain.

Testing Yogurt
Day 2, Lesson 1

Air out the insulated box before the lesson begins; the box exudes the pleasant smell of ripe yogurt. Ask the students to sniff the box in groups. The reaction may be sobering, for example, "Uuh, it smells like sour milk!" Students may ask facetiously whether they may go to the toilet. Others will express their

revulsion with laughing faces, and their cheerfulness may last for the whole of the next lesson.

Do not allow the students to try the yogurt until they examine it visually; the students should also consider how yogurt was formed from milk. Precise observation should precede any attempts at interpretation. Observations will vary: "It is thick but still a bit runny." "It has been sweating!" (interpretation); "There is a layer of fat at the top" (interpretation); "Like blancmange."

How can this be explained? "The milk has got thicker" (observation); "The lactic acid bacteria have got bigger" (interpretation). Allow the students to try their yogurt; return to the question later.

The hope that all will exclaim "delicious" is now very slight, as the following opinion gradually predominates: "It doesn't taste of anything, it's just sour!" (figure 27). The teacher's inquiry will generally reveal that the students only eat yogurt with fruit, sugar, or cream. Nonetheless, some will eat all of their yogurt, and some will have to be stopped from eating what the others leave. This is necessary because an excess of lactic acid bacteria may suppress intestinal bacteria, resulting in diarrhea. Discussion generally reveals that the students' taste is greatly affected by the sugar and fat content of their normal diet. Students may also prefer yogurt that is produced in a normal "yogurt machine" (three hours at a constant temperature of 42°C). Such yogurt has a better consistency and is milder than the yogurt from the insulated box (overnight, starting temperature 45°C).

After the milk has been tested, ask the students to explain why the milk thickened. Squeeze some lemon juice into a jar as a demonstration experiment. The thickening of the milk is attributed to the lemon juice. Like this acid, the acid produced by the lactic acid bacteria thickens the milk. Expand upon the information written on the blackboard the day before. At the end of the lesson the students observe that the milk and lemon juice, which initially showed only flocculations, now look like yogurt.

J2. Production of Sauerkraut

Prerequisite Biological Knowledge

No special knowledge is required.

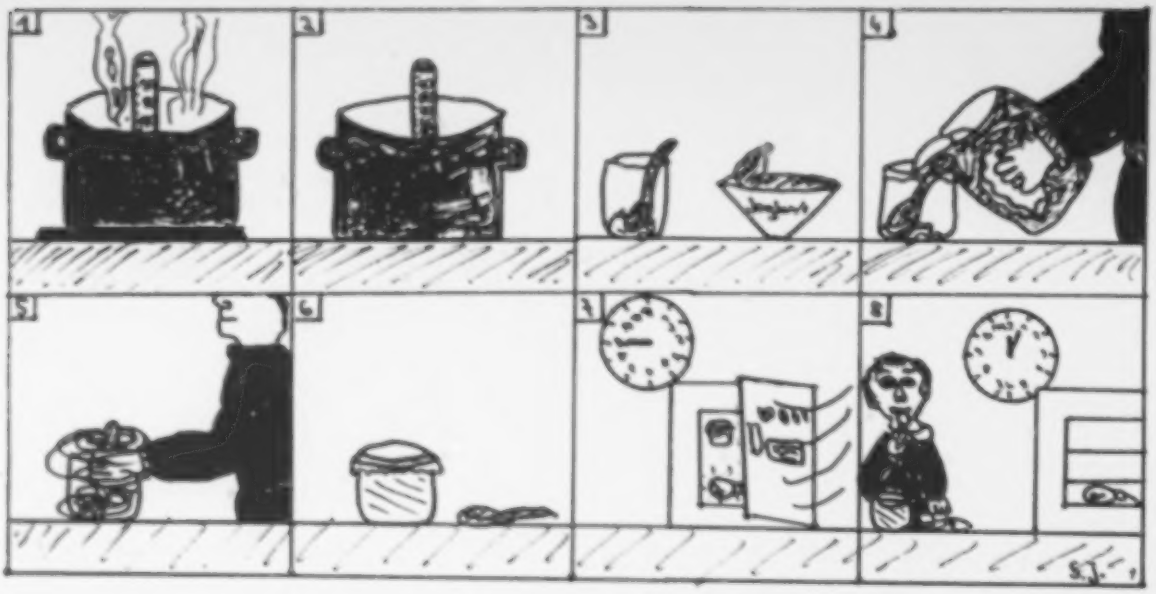

Figure 26. Equipment and procedure for making yogurt (a student's drawing).

Figure 27. "It doesn't taste of anything, it's just sour!"

Equipment

1 bowl (diameter: 30 cm)
chopping board
kilner jar (2 ml) with rubber ring, lid, and clasp
4 kitchen knives
1 wooden cylinder (4-5 cm) or 1 egg cup

Materials

1 large cabbage
20 - 40 g salt

Procedure

1. Cut a white cabbage into strips on a chopping board.
2. Put the chopped cabbage into a bowl, together with the salt.
3. Mix together well the cabbage and the salt.
4. Put the salted cabbage into a kilner jar.
5. Press the cabbage together well with your fist.
6. Press a wooden cylinder or an egg cup onto the cabbage, attach the lid, and close the kilner jar with a clasp. Tell the students that the cabbage must remain like this for about two weeks, until sauerkraut has been formed. They can try the sauerkraut at that time.

Begin the lesson by telling the students that they are going to make sauerkraut. "Delicious!" Most of the students will already be looking forward to trying it later. While some of the children finely chop the leaves of a cabbage (without stem) (figure 29), the rest of the class should consider bacteria in general. Encourage the students to ask questions. Teachers should expect questions such as the following: "What do they look like?" Outline diagrams show that some bacteria look "like ships' propellers" (figure 30A).

"How fast are bacteria?" Bacteria can move at the rate of several cm per hour. Encourage the students to make comparisons with their own (high) speed on a bicycle.

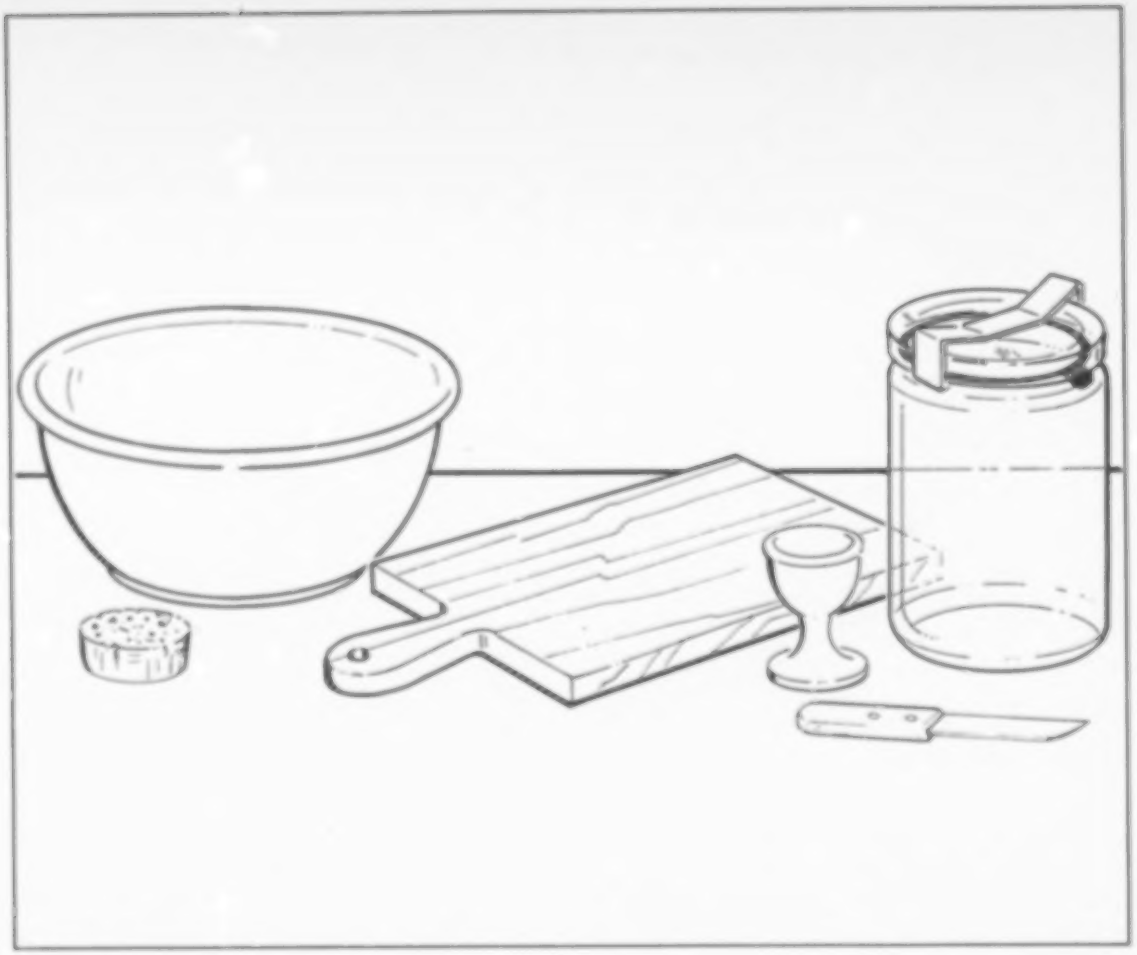

Figure 28. Required equipment.

"What do they eat?" The answer is complicated. Bacteria do not have a mouth, they let a little milk in through their entire surface. Support the answer with an outline drawing on the blackboard.

"Do they pee?" Use the outline sketch again to demonstrate that bacteria do not produce urine. Lactic acid bacteria produce lactic acid, which they exude through the whole of their surface.

"Do the bacteria live in a house?" Explain that lactic acid bacteria swim around freely in the milk. "Can they die?" Bacteria can be killed. If they are lucky, they do not

Figure 29. Chopping the cabbage.

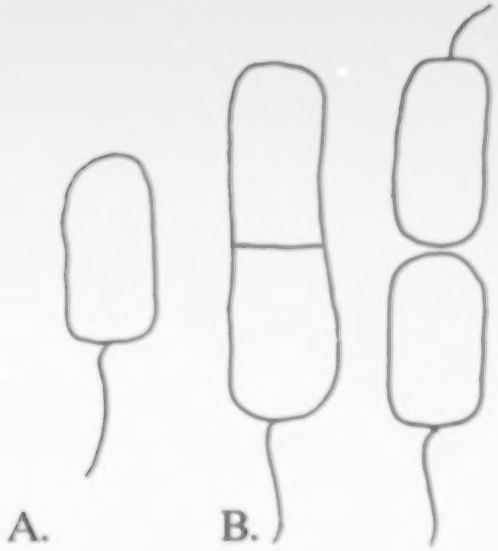

Figure 30. A. Outline diagram of a bacterium; many bacteria look like this. The process is used for motion; it works like a ship's propeller. B. Division of a bacterium; first it gets longer, then it divides in the middle.

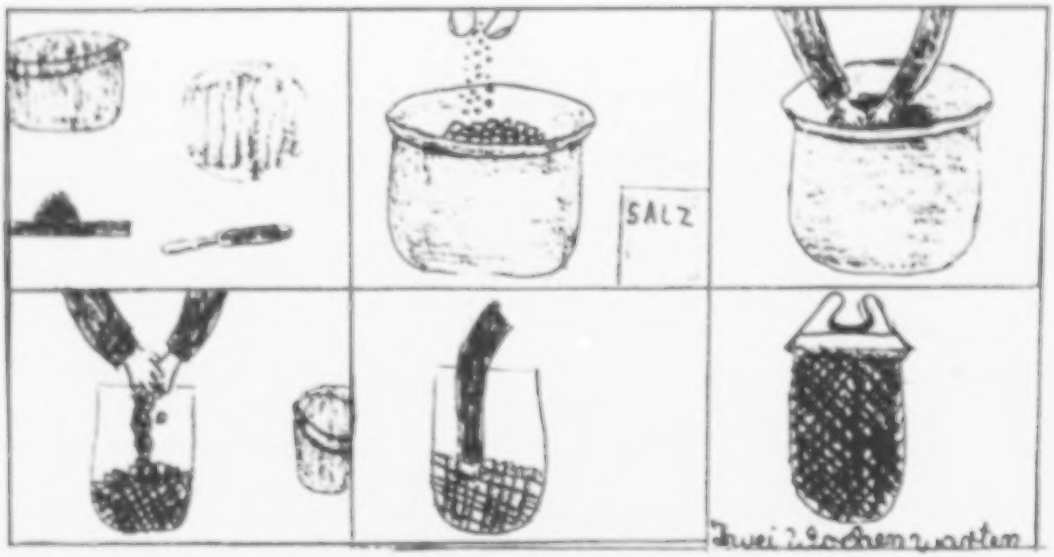

Figure 31. Equipment and procedure for making sauerkraut (a student's drawing).

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have to die. Instead, when bacteria reach a certain age they simply divide into two offspring, leaving no trace of the parent behind. Use an illustration similar to figure 30B to show how two bacteria are produced from one by division.

"How big are they?" Review the homework assignment in which the students estimated how many bacteria fit onto the edge of a sheet of paper. Most student estimates are between a hundred and a thousand and are, by chance, roughly correct. To a certain extent the values are higher. You can extend the explanation as follows: If you divide a millimeter into ten equal parts, then each part is as thick as a piece of paper. If you could cut the sheet of paper into a hundred parts, each part would be about as thick as a fairly big bacterium.

By this time, the students should have finished chopping the cabbage. The chopped cabbage is now salted (salt extracts the juice). Mix salt and pieces of cabbage leaf in the bowl by hand, then gradually press the mixture into a kilner jar. A 2-liter jar can almost accommodate a large cabbage about 30 cm in diameter. Use 2 - 2.5 g of salt per 100 g (after Präve, 1982, pg. 256). Encourage the production of juice by pressing the cabbage down with your fist. This will produce liquid. There should be a surplus approximately 1 cm high above the cabbage. Press a wooden cylinder (4 cm high, 5 cm in diameter) or an egg cup between the lid and the cabbage and seal the lid with a rubber seal. Place the filled and sealed jar in the cupboard. After a fortnight, open the jar for the first time to test whether sauerkraut has been produced from the cabbage.

Review the following information with the students: Sauerkraut bacteria make acid from a small amount of cabbage. The acid causes the acidic taste; it also stops the sauerkraut from going bad--from becoming moldy.

As homework, the children should describe the procedure for making sauerkraut and should draw the materials and apparatus required (figure 31). At least two weeks after the lesson, have the children bring plates and forks into school to try the sauerkraut. They may be even more enthusiastic about this than about eating the yogurt. Some may make sauerkraut at home.

Investigation K. **The Formation of Lactic Acid in Sourdough - A Model for the Perfection of Bacterial Cultures (by E.R. Lucius & L. Rohweder)**

The natural method of making bread dough sour--invented by the Egyptians--has been in use for 3,500 years. The Egyptians observed that dough made from rye flour begins to ferment in certain conditions, and they discovered that this sour-tasting dough could be used to bake light piquant bread. They also knew that one could produce large quantities of sourdough with a small amount of sourdough, and they therefore always saved a small amount of dough.

The souring of the rye flour is caused by consecutive fermention of the dough by two groups of microorganisms: yeasts and lactic acid bacteria. Yeasts of the genera *Saccharomyces* and *Khyveromyces*, together with lactic acid bacteria of the genera *Lactobacillus* and *Lactococcus*, stick to the grain and get into the flour in this way.

Sourdough is made by mixing rye flour with water. The organisms take up their activity and enrich the "dough" in their substrate. Repeat inoculation of fresh dough with this culture encourages the yeasts to grow first; the lactic acid bacteria come to the fore later. During the growth of the yeast, the volume of the dough greatly increases and the dough smells of alcohol. After the third inoculation, one can measure the souring of the dough (pH 4.5). The sourdough ultimately contains predominantly lactic acid bacteria.

Prerequisite Biological Knowledge

knowledge of pH-values
lactic acid bacteria and yeast

Equipment

aluminum foil
1 measuring cylinder, 100 ml
1 felt-tip pen, waterproof
6 glass beakers, 400 ml
1 shallow plastic bowl, 15x30 cm
1 set of scales
1 spatula
1 thermometer (50°C)
1 wooden spoon

Materials

rye flour, type 1250
warm tap water (40°C)
pH paper (3.5 - 5.5)

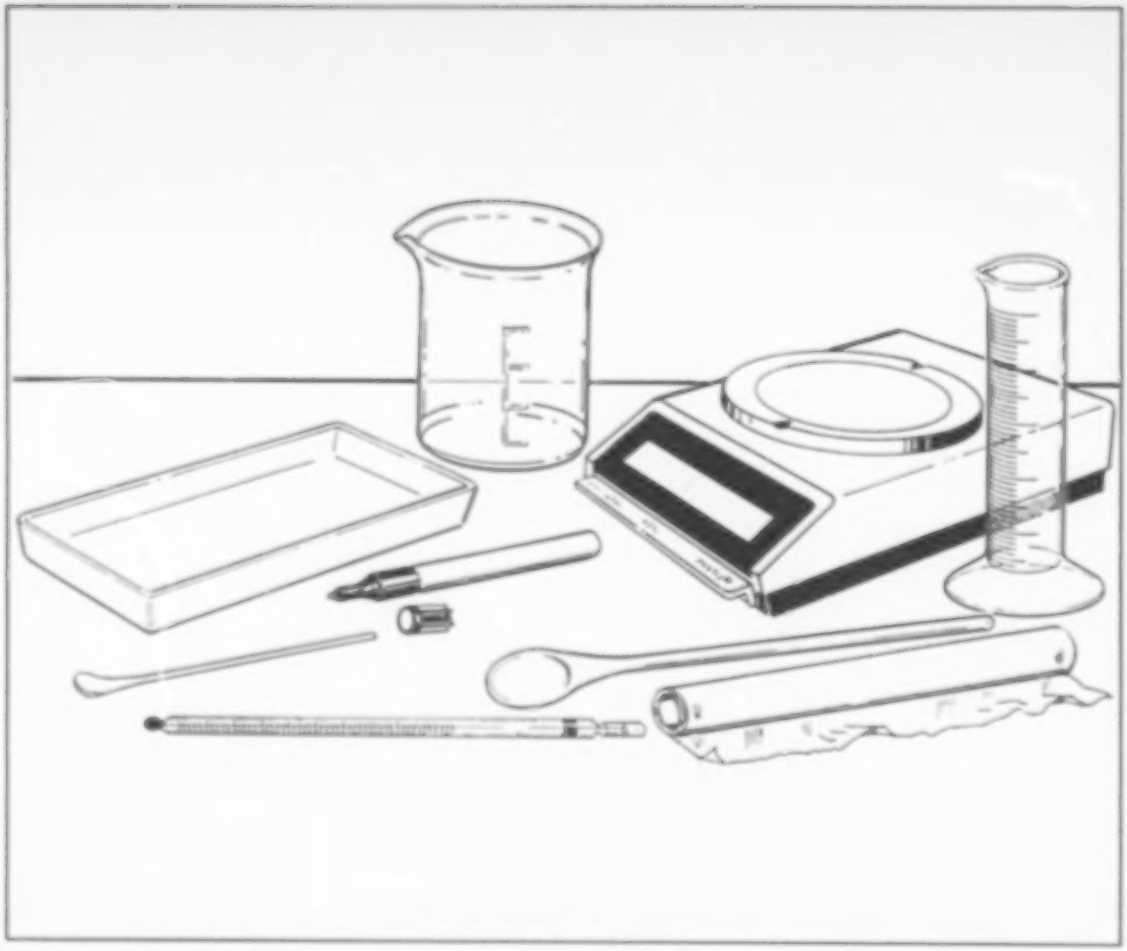

Figure 32. Required equipment.

Time Requirements

mixing of the dough: 15 minutes
inoculating: 2 x 5 minutes waiting time: 3 x 24 hours

Procedure

1. On the first day, mix dough made from 100 g of rye flour and 100 ml water (40°C) with the spoon. Put the dough into the first glass beaker (dough 1); seal the beaker with aluminum foil and place it in a safe place at room temperature.
2. On the next day, prepare another dough as described in step 1; put it into a glass beaker (dough 2). Mix a quarter of dough 1 (from the day before) with 75 g of rye flour and 75 ml warm tap water (40°C). Place this into a glass beaker (dough 3). Seal the glass beakers (dough 2 and dough 3) with aluminum foil and place them in a safe place. The rest of dough 1 is no longer required and can be put on the compost heap.
3. Three glass beakers are required on the third day. Fill the first with fresh dough prepared as described in step 1 (dough 4). Place a mixture of 75 g rye flour and 75 ml water (40°C) into the plastic bowl (which has been washed); add 50 g of dough 2. Place this mixture into the second glass beaker (dough 5). Finally, place a mixture of 75 g rye flour and 75 ml water (40°C) into the plastic bowl (which has been washed); add 50 g of dough 3. Place this mixture into the third glass beaker (dough 6). Seal the three (dough 4, dough 5, dough 6) glass beakers with aluminum foil and put them in a safe place. Doughs 2 and 3 are no longer required and can be put on the compost heap.
4. After three hours, measure the volume of doughs 4, 5, and 6, appraise the smell, and measure the pH with pH paper.

Explanations and Suggestions

Step 1. Spontaneous growth of the bacteria contained in the flour requires the addition of sufficient water of the right temperature (40°C), and standing time. Constant humidity and temperature (20°C) also are necessary. The lactic acid bacteria can develop their activity in the rye flour particularly well because rye contains very little gluten protein, in contrast to wheat. Dough made from wheat flour only "ferments" if baker's yeast is added to it.

Steps 2 and 3. Fresh dough is prepared repeatedly because sourdough of various ages should be available for comparison on the third day.

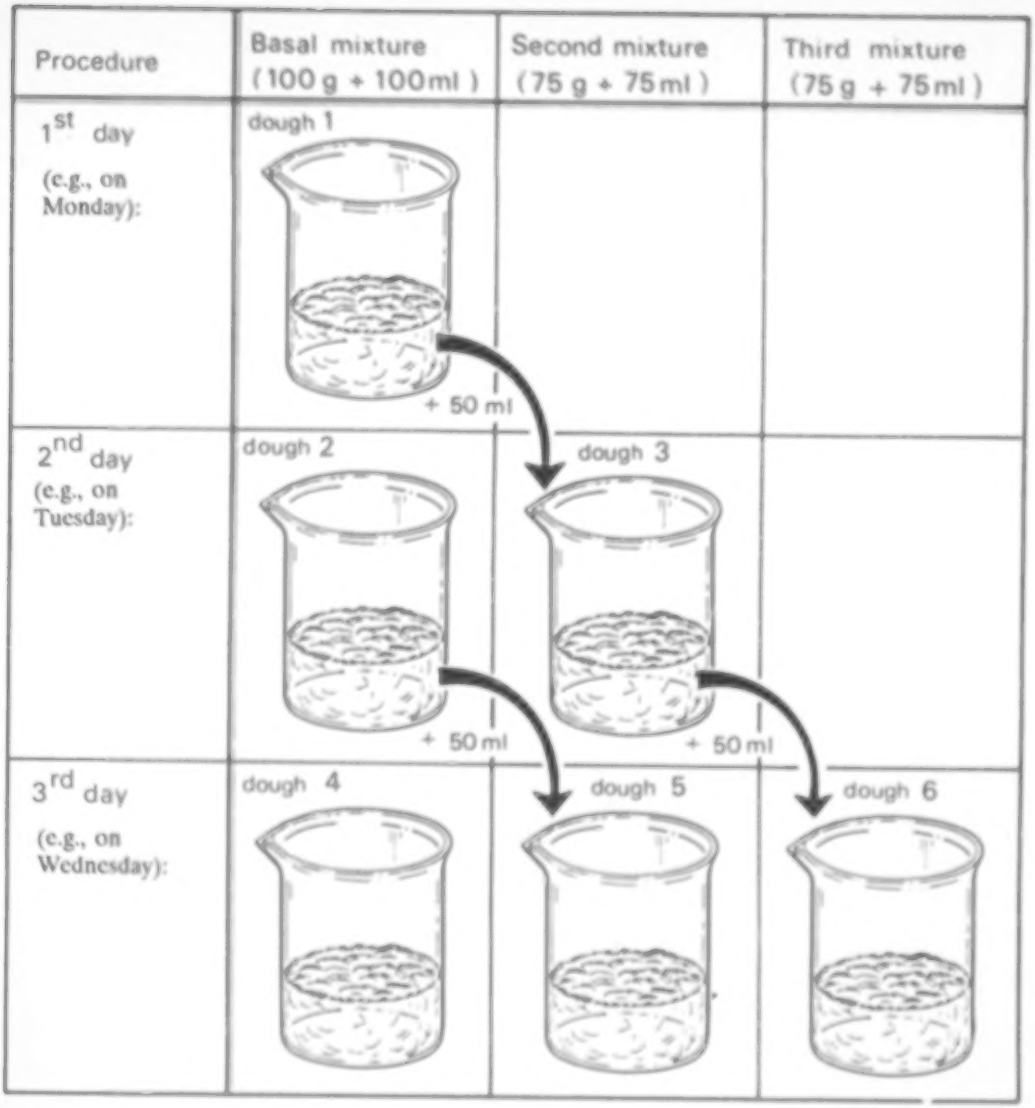

Figure 33. Making sourdough in glass beakers.

Problems and Questions

1. Why is water of the right temperature essential? (The dough being prepared requires a specific temperature to promote the growth of yeasts and lactic acid bacteria.)
2. Why does dough 5 take up more space than dough 4 and dough 6 when the pH continually increases from dough 4 to dough 6? (The increase in the volume of the dough can be traced primarily to the activity of the yeast cells, that is, formation of carbon dioxide. The growth of the yeast is reduced as the dough becomes increasingly acidic. The lactic acid bacteria become increasingly more enriched after the dough has been inoculated several times.)

Investigation L. Wine from Juice, Vinegar from Wine--A Model of Downstream Processing from Glucose to Acetic Acid (by E.R. Lucius)

The production of alcohol and vinegar are biotechnical procedures that may well have been in use for more than 6,000 years. Underlying both procedures are microbic reactions of microorganism that are quite varied phylogenetically and metabolically.

In learning to produce wine, students learn a classic procedure by which microbic conversion can be experienced via the rapid fermentation process and later through the alcoholic smell of the must. The fermentation of vinegar, on the other hand (for which the home-made wine can also be used), represents a modern biotechnical procedure in which the central focus is the construction of a continuous bioreactor. By linking both processes, glucose is converted in stages from glucose to acetic acid via the intermediate stage of ethanol.

1. The production of wine
   The types of yeast that cause alcoholic fermentation belong to the genus *Saccharomyces*; they can always be isolated from ripe fruit. Nowadays, the production of wine employs certain strains of *Saccharomyces ellipsoideus*, which is closely related to the brewer's or baker's yeast *S. cerevisiae*. During this process, the fruit sugar is converted to ethanol and carbon dioxide.

2. The preparation of vinegar
   Certain bacteria, especially of the genera *Acetobacter* and *Gluconobacter*, can oxidize ethanol to acetic acid. In this process, ethanol is oxidized to acetic acid via intermediate

stages. In the past, vinegar was produced at home. An industrial procedure for the production of vinegar was developed in the fourteenth century in the area of Orleans, France: one part of mash and one part of fresh wine vinegar were put into wooden casks, which were lying on their sides, as a "starter." In later techniques, the vinegar bacteria were placed onto wooden lattices or beech shavings to encourage them to expand. These techniques (Fessel procedure) were eventually developed to such a degree that a solution containing alcohol was dripped onto the container that was filled with beech shavings from above, while a counter current of air was guided over the shavings from below (figure 35).

L1. Production of Cider

Prerequisite Biological Knowledge

respiration
fermentation
aerobic and anaerobic microorganisms
knowledge of baker's yeast and wild yeasts

Equipment

1 rubber tube, internal diameter 5mm
1 rubber bung with air lock
1 household funnel
1 demijohn, 2 liters
All materials are available from the chemist.

Materials

250 g granulated sugar
2 x 0.7 liter bottles of unclarified apple juice
1 package of wine yeast (from the chemist)

Figure 34. Measuring the volume of the dough.

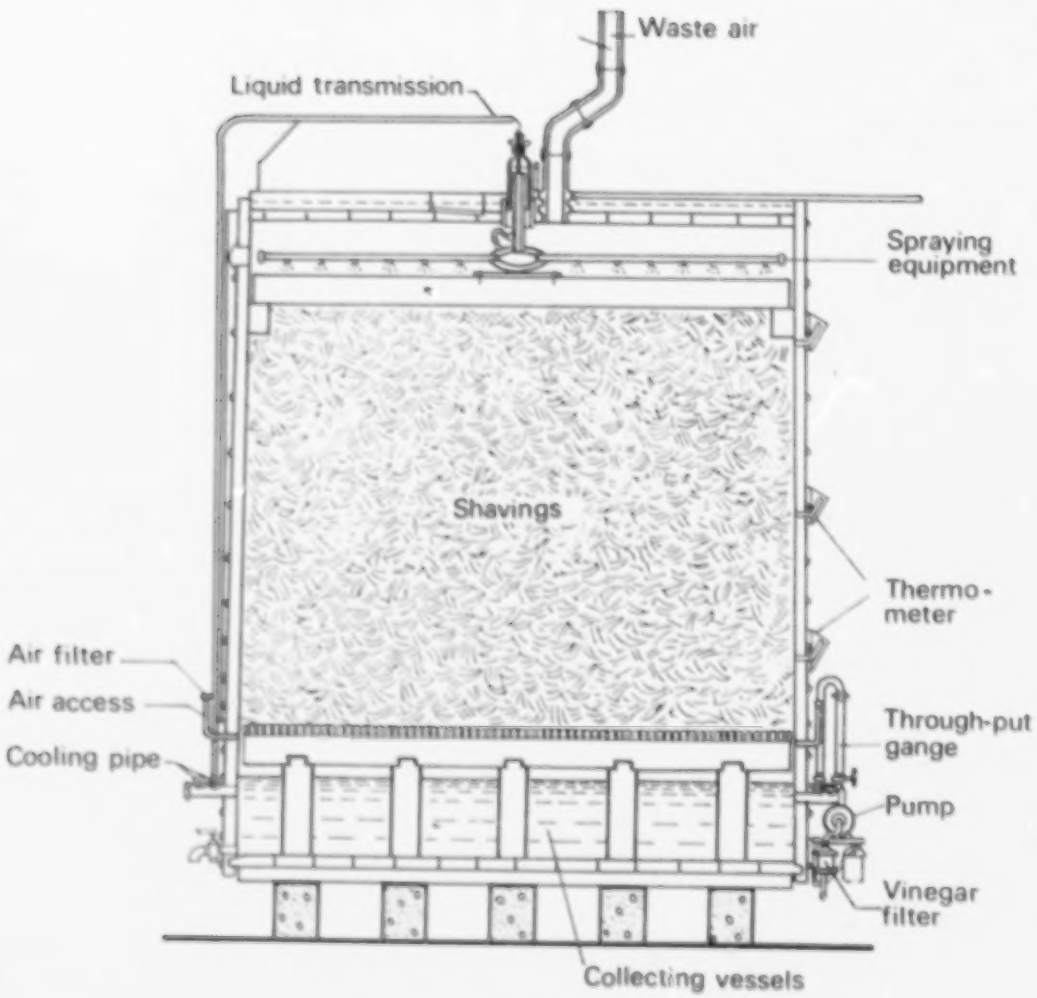

Figure 35. FRINGS - vinegar generator.

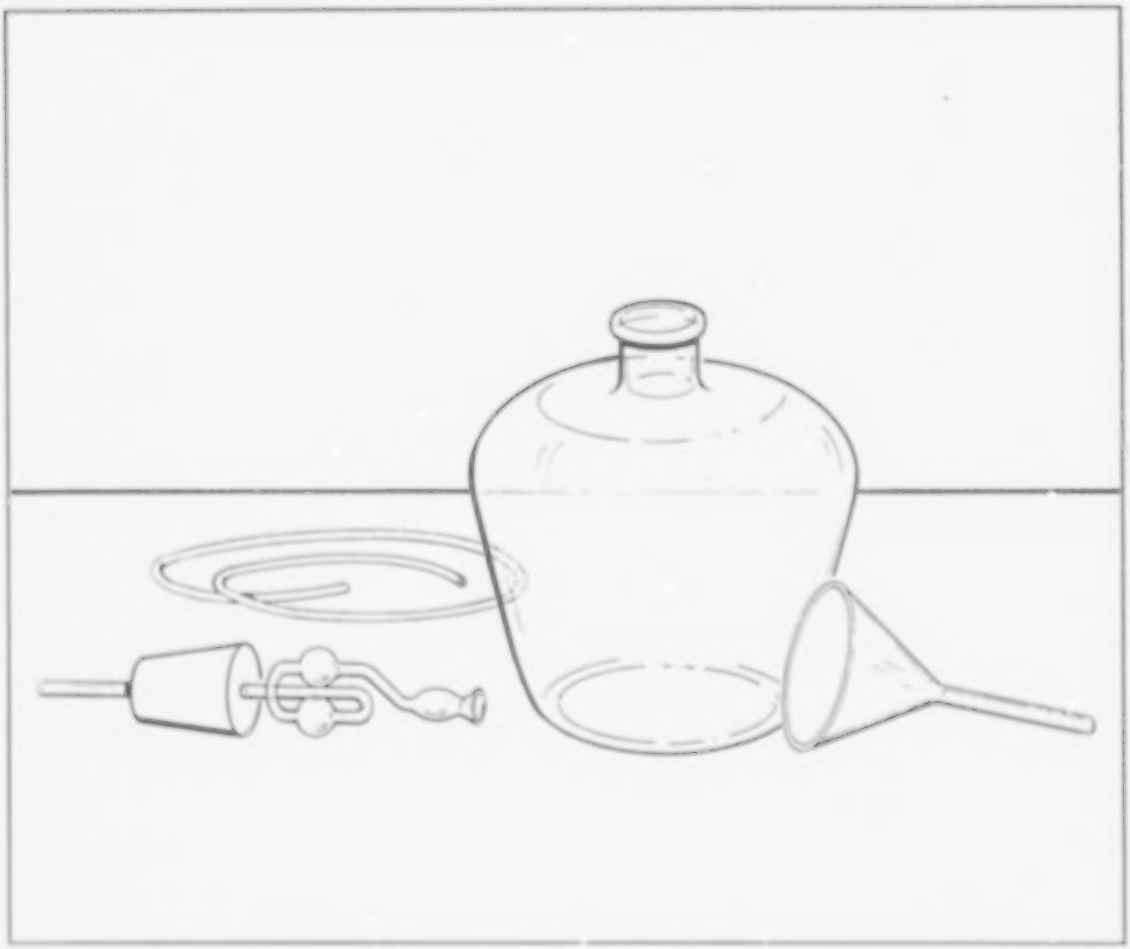

Figure 36. Required equipment.

Time Requirements

starting and inoculating the wine: 5 minutes
fermentation time: 6 months

Procedure

1. Place 240 g granulated sugar into the demijohn (dry funnel); add a bottle of apple juice. Dissolve the sugar by carefully swirling the demijohn from side to side.
2. Add the wine yeast with the second bottle and swirl it round.
3. Seal the demijohn with a rubber bung and an air lock that is filled with water.
4. A cloudy development in the fermentation gases is visible after three days. Vigorous fermentation recedes after approximately ten days.
5. After about six months the yeast has sunk to the base and the fresh wine appears clear. Use a piece of rubber tubing to siphon the wine off from the yeast.

Explanations and Suggestions

Step 1. The alcohol content determines the life of wine to a large degree. Table wines with an alcohol content of 8 percent by volume generally have to be preserved by the addition of sulphurous acid or potassium pyrosulphate (for example, against vinegar bacteria), while port--with 15 percent alcohol by volume--preserves itself. Sugar must be added to achieve a high concentration of alcohol (cider as port). Cider can be made from industrially produced apple juice in an extremely simple way, as it contains almost no pectin, but sufficient acid. Pectins might cause the fermenting wine to set or might prevent the deposition of particulate matter. Wines that contain very little acid (for example, pear wine) often taste insipid and do not produce the esters necessary for good bouquet. There is, therefore, no need to clarify the wine to remove particulate matter that is linked to the pectin or to acidify the wine artificially. Neither is the addition of yeast nutrient salt (which contains nitrogen) necessary, as apple must contains sufficient nitrogen compounds.

Step 2. At the beginning of the fermentation process, the respiration processes of microorganisms creates negative pressure in the demijohn. This must not be allowed to last for longer than three days. If the yeast culture does not grow, the juice must be inoculated again.

Problems and Questions

1. Why should pure culture yeasts be used for wine making? (The fermentation of wild wine yeasts involves the danger of unpredictable and undesirable effects that can spoil the fermenting wine.)
2. How do port wines preserve themselves? (The high alcohol content has a

disinfecting effect.)

3. Why must the demijohn not be filled to the top? (The carbon dioxide produced by the fermentation of alcohol can form several liters of foam together with the yeast cells. These can be pushed through the air lock and out of the demijohn. As a safety precaution, the demijohn should never be kept on a surface that must remain clean.)
4. What is the air lock for? (During fermentation, the formation of carbon dioxide creates excess pressure in the demijohn. The water contained in the air lock prevents large amounts of oxygen from entering the demijohn and encouraging the growth of vinegar bacteria.)

L2. Continuous Production of Vinegar

Prerequisite Biological Knowledge

respiration
fermentation
aerobic and anaerobic microorganisms
knowledge of baker's yeast and wild yeasts
fermentation of acetic acid

Equipment

aluminum foil
1 glass tube
1 aquarium pump
1 glass tube, right angled
1 one-way tap, right angled
2 pipettes, 5 ml, sterile
1 conical flask, 500 ml pipetting aids
1 culture tube
1 rubber bung, single bored
2 glass bottles, 1.5 liters with stopper attachments at their bases
1 rubber bung, double bored
rubber tubing
stand material

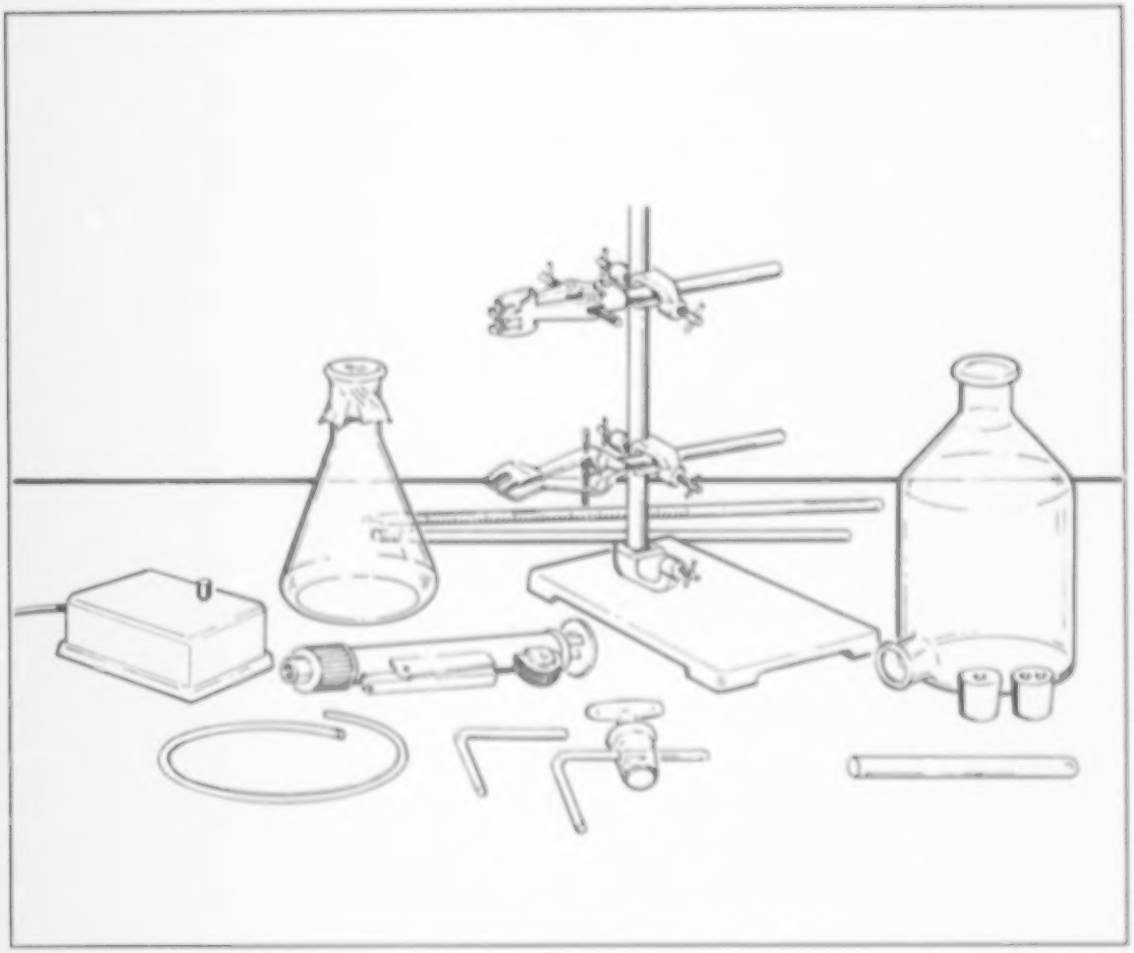

Figure 37. Required equipment.

Materials

pure culture of *Acetobacter aceti* (DSM-No. 3508)
beech shavings (possibly get a carpenter to rasp some beech strips)
cotton wool
4distilled water
sterilized distilled water, 750 ml
wine (cider or unsulphured port), 250 ml
1 n NaOH

Time Requirements

preparation and autoclaving of the solutions: 45 minutes
preparation of the culture: 15 minutes
waiting time: 48 hours
construction of fermenter and preparation of main cultures: 45 minutes

Preparation

Resuspend the culture of *Acetobacter aceti* according to the manufacturer's instructions; inoculate the culture with 100 ml medium for the cultivation of vinegar bacteria in a 300 ml conical flask (see appendix). To ensure sufficient addition of oxygen, place the flask on to a magnetic stirrer for 48 hours (the stirring rods should be autoclaved with the culture medium before use).

Procedure

1. Attach a 1.5 liter bottle that has a fixture for a bung at its base about 40 cm above the table, using the stand. A single-bored rubber bung with an angled, one-way tap seals the lower outlet of the bottle where the bung is attached.
2. Attach a second bottle of this kind directly beneath the outlet of the one-way tap ("fermeter"). Seal its lower outlet with cotton wool inside; fill its interior with beech shavings.
3. Close the lower outlet of the second bottle with a bung that has been bored through twice. In one of those openings, attach a glass tube as an attachment for the aquarium pump. In the other, insert a right-angled glass tube as a product outlet.
4. Use an aquarium pump to blow air constantly into the inside of the fermenter through the bung; the cotton wool filter keeps the system sterile.
5. As medium, use a mixture of unsulphured port and sterile distilled water in the ratio of 1:4. The pH value must be adjusted to 7.0 using 1 n NaOH. Pour 200 ml of the medium over the beech shavings in the fermenter. Allow the contents to stand for 48 hours.
6. Place the other 800 ml of the medium in the upper container. Adjust the tap so that it releases one drop per 5 minutes.
7. The product is continuously caught in a 500 ml conical flask at a rate of 1 drop per five minutes. Test the product once a day with indicator paper to monitor the development of acid.

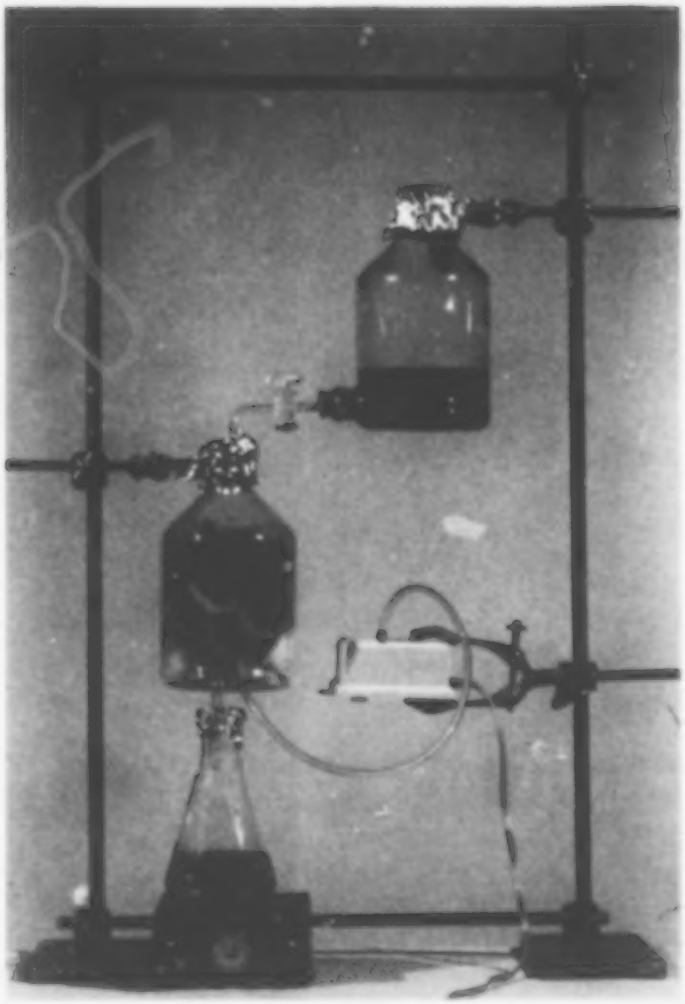

Figure 38. School fermentation of acetic acid.

Explanations and Suggestions

Step 2. The beech shavings immobilize the vinegar bacteria to the fermenter. The cotton wool should retain coarser particles that can be separated from the wood shavings.

Step 5. The wine must be unsulphured so that the vinegar bacteria do not die off. This is the case in homemade wines and is usually true of ports, as well. The pH value of the medium must be adjusted to 7.0 so that the reduction of the pH value due to the formation of vinegar can be monitored.

Problems and Questions

1. Why is air blown into the bioreactor? (Without oxygen, the vinegar bacteria would die off.)
2. Why must the air be filtered so that it is sterile? (The air in the room contains fungal spores that develop in the fermenter and may cause the formation of mold on the beech shavings.)

2. ***Fermentation Processes that Occur in the Degradation of Waste Products--The Degradation of Cellulose.***

In Brazil, bioethanol has been produced for many years from cane sugar molasses, a waste product in the production of raw sugar. Bioethanol is used as a fuel for vehicles throughout the country.

The microbic decomposition of cellulose waste products from the paper industry or from sugar factories is useful in two ways: the burden on the environment (eutrophication of rivers and the pollution of the air with unused CO_2 through dissimilation processes) is reduced considerably, and energy extracted from the products of decomposition (bioethanol, biogas) is fed back into the industrial process.

Investigation M. **The Microbial Decomposition of Cigarette Paper (by E.R. Lucius)**

Prerequisite Biological Knowledge

the ecological significance of microbic decomposers

the enzymatic decomposition of cellulose

Equipment

1 autoclave or pressure cooker
2 glass petri dishes
1 large dish that can be covered as a damp chamber
1300 ml conical flask with cellulose bung
1 drying cupboard (or a bunsen burner, tripod, pipeclay triangle, and crucible)

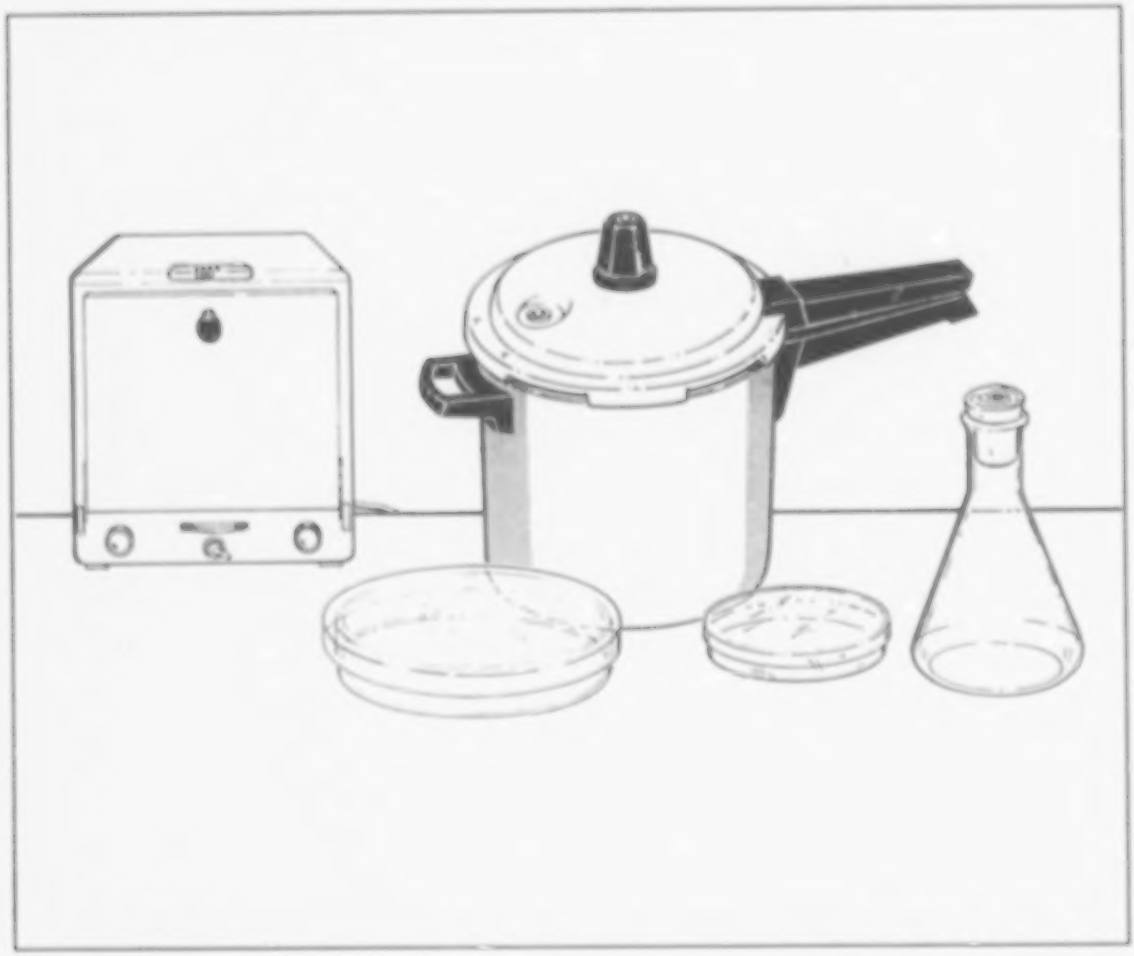

Figure 39. Required equipment.

Materials

80 g soil
blotting paper (approx. 90 cm^2)
sterile tap water
6 strips of cigarette paper

Time Requirements

sterilization of the water: 30 minutes
sterilization of a part of the soil sample: 180 minutes
preparation of the experiment in the damp chamber: 15 minutes
waiting time: about three to four weeks

Preparation

Sterilize 100 ml tap water in a closed conical flask for 30 minutes. Sterilize half of the soil sample in a glass petri dish in a drying cupboard at 180°C for three hours, or use a bunsen burner, tripod, pipeclay triangle, and crucible for 30 minutes.

Procedure

1. Place the unsterilized part of the soil sample into a glass petri dish. Dampen the sterilized soil sample with sterile tap water in the other sterile petri dish. Ensure that both of the experiments in preparation are approximately equally damp.
2. Place three strips of cigarette paper--approximately 1 cm wide--on the dampened soil sample in each of the petri dishes.
3. Cover both petri dishes, seal the edges with adhesive tape, and place them into the larger dish, which has been covered with dampened blotting paper (damp chamber: "Feuchtekammer"). Cover the large dish.
4. Allow the experiment to stand in a safe place for about four weeks.

Explanations and Suggestions

Step 1. The sterile soil serves as a control; no microorganisms should grow on the cigarette paper during the four weeks.

Step 2. For health reasons, cigarette paper does not contain lignin and is therefore more suitable for this investigation than is filter paper, which does contain lignin. Lignin prevents enrichment of organisms that decompose cellulose.

Step 3. The petri dishes must be sealed so that microorganisms do not accidentally escape; there is no guarantee that dangerous microorganisms will not develop. The damp chamber prevents the soil from drying out.

Problems and Questions

1. Why can only microorganisms that decompose cellulose be enriched on cigarette paper? (Cellulose is the only source of carbohydrate in the paper. Microorganism that grow on the paper also live off cellulose).

Figure 40. Cellulose decompositions of cigarette paper in a damp chamber.

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Enzyme Technology

How can orange juice be transported economically from Spain to Germany? What about transporting juice from tropical fruits such as mangos, papayas, and passion fruit from South American to the U.S.A.? The juice is concentrated in the land of origin and the water that has been removed is replaced in the country where it is to be consumed. This method reduces transportation costs.

There is a snag, however; because fruit juice contains pectin, jelly usually forms when fruit juice is concentrated. A great deal of the pectin is contained in the juice of fleshy fruit such as papaya. When water is removed, pectin polymerizes and causes setting. This is prevented in the fruit-juice industry by adding pectinase, an enzyme that splits pectin. A form of pectinase can be extracted from the mold *Aspergillus niger*. Its use in the production of juice also increases the juice yield because a large proportion of juice is bound by pectin and retained in the marc.

Many other enzymes play an important role in industry; for example, amylase, which is also extracted from mold, is used in the textile industry to remove starch from cotton. Starch naturally adheres to cotton and inhibits the uptake of dye when textiles are being dyed. The baking industry mixes amylase with flour and supplements the naturally occurring amylase in flour. This enzyme is necessary for the preparation of dough because it breaks down a small proportion of the starch in the flour to glucose, which serves the yeast as food. Yeasts cannot produce amylase.

Even manufacturers of liquid and powder detergents use amylase. It is used to break down the starch that forms as dirt on cutlery or in clothes. Protease is used in washing powder (except in wool-washing powders); it decomposes protein stains. Lipases are used to break up fat stains. Cellulases are used in the processing of fruit and vegetables; they destroy the cell walls, which are composed of cellulose.

Cellulases, amylases, proteases, and lipases are enzymes that are released by cells into the environment to help break down the high-polymer food molecules that cannot be taken up into the cells whole. Lactase, on the other hand, works inside the organism, where it decomposes the relatively small dimerous lactose molecules to alpha glucose and beta galactose.

Whey contains relatively large quantities of lactose. At one time, this was sent back to farmers, who used it as cattle fodder. The construction of large dairies far away from the farms, however, made the distribution and transport costs too high for whey to be used in this manner. Whey is now a waste product that must be devoid of lactose before it can be released into rivers.

Many adult humans cannot break down lactose in the digestive tract because they no longer produce the "infant's enzyme," lactase. Undigested lactose removes water from the intestinal wall, which results in diarrhea. Bacteria in the intestinal flora that can split lactose decompose the products of splitting, developing gas in the process; the gas causes flatulence. In Europe, where milk has been an important part of the adult diet for a very long time, lactase insufficiency is far less widespread than in other parts of the world. Lactase is used to decompose lactic acid and to produce glucose.

In the following investigations, the students describe the effect of pectinase, explore the splitting of lactose, and decompose starch with *Bacillus subtilis*.

1. ***Experiments Relating to Pectinase in the Industrial Production of Juice.***

Pectins are vegetable polysaccharides; their main components are galacturon acid and its methylester. The multiplicity of pectins is determined by the various degrees of polymerization and esterification. Together with cellulose, they are reticulum substances of vegetable cell walls, especially as a sort of "putty" in the intermediate lamella between the cells. They occur in solution in the cell sap.

Pectins have a great ability to combine with water, which accounts for the high gelling capacity of jams and jellies. For this reason, pectins are extracted from slices of sugar beet and from the remains of apples and lemons that have been used for making juice. They are then used as gelling agents in the food, cosmetic, and pharmaceutical industries, and in medicine.

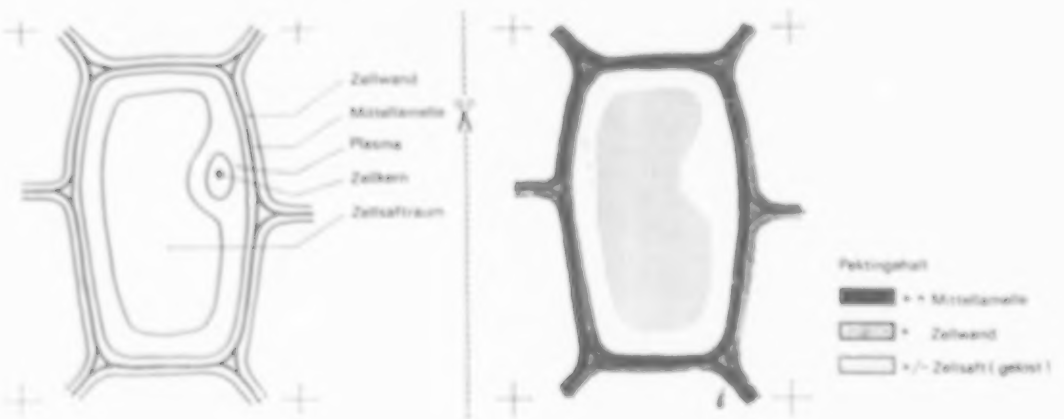

Figure 41. Pectin as a component of fruit cells.

Pectinases destroy the pectin in the cell wall and in the plasma so that it no longer retains juice in the chopped fruit. Fruit can therefore be pressed more effectively, resulting a high yield of juice.

Pectinases also are used to clarify fruit juice. Pectin retains substances that make the juice cloudy. Once pectin has been destroyed, those substance can easily be precipitated out. Students will examine the production of apple juice to investigate the use of enzymes in the food industry. The students also should learn that a great variety of enzymes is used in the food industry, and they should receive information about enzyme technology, an area of modern biotechnology. Last, the students should be able to understand the information provided by juice manufacturers on bottle labels, and so be more knowledgeable consumers.

Investigation N. **Why Does Apple Juice Gel When It is Boiled? (after Ch. Labahn-Lucius, 1990)**

Begin by asking the students to bring apple jelly and clear and cloudy apple juice to school. Ask general questions about the production of these products.

Write the following question on the blackboard: "What sets the jelly?" To determine the answer, the students (in groups) make jelly from apple juice. The teacher should produce the juice in advance, using the method described below (see procedure, steps 1 and 2). The addition of sugar causes the students to suspect that this causes the gelling. This suspicion is disproved by boiling a pure sugar solution. Refer to "substance X," which causes gelling, as pectin. The students discuss why pectin (in Europe known as Opecta) has to be added to certain types of fruit for the production of jelly. Figure 41 shows that pectin is a component of the cell.

Prerequisite Biological Knowledge

No special knowledge is required.

Equipment

1 glass beaker (800 ml)
1 chopping board
2 glass beakers (400 ml)

1 watch glass
1 tripod
1 plastic bowl
1 ceramic net
1 piece of muslin
1 bunsen burner
1 gas lighter
1 wooden spoon
1 kitchen knife

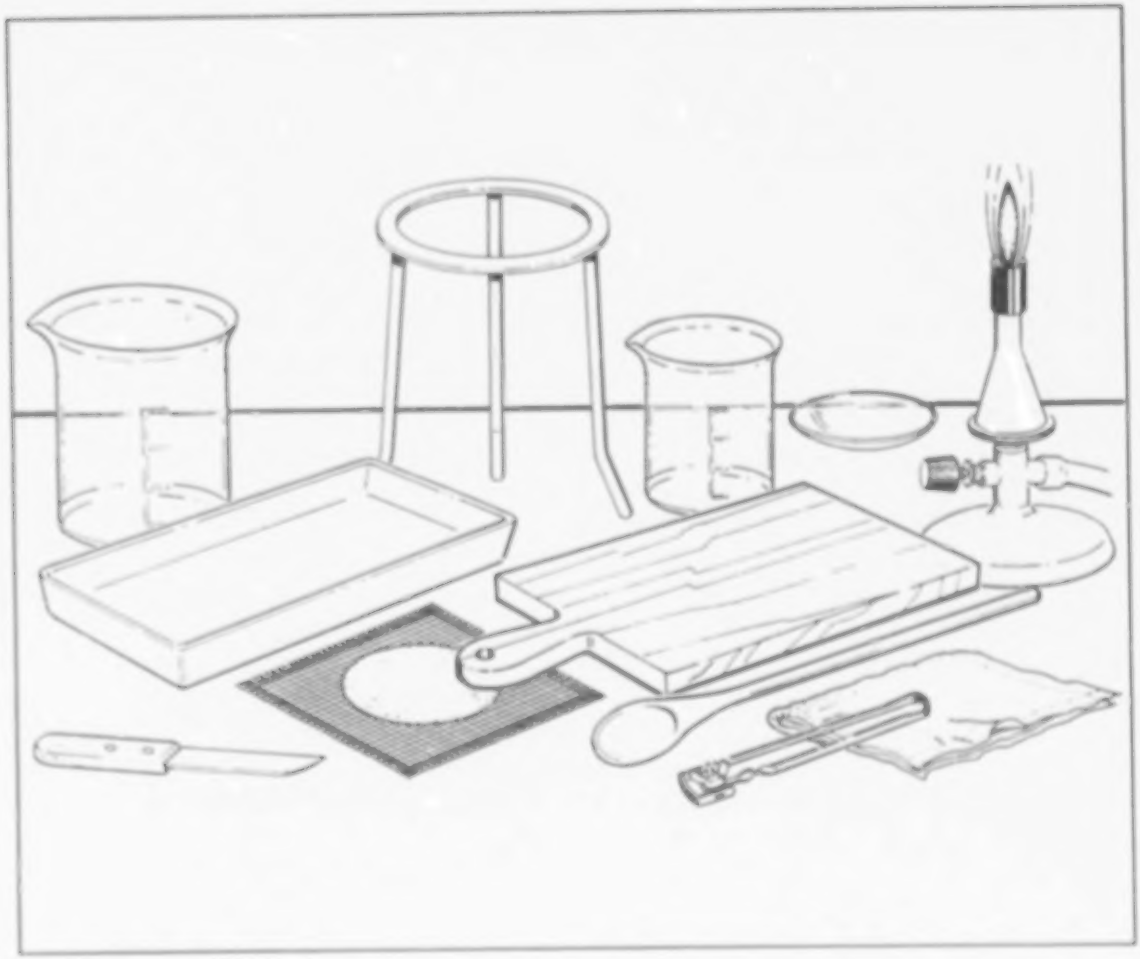

Figure 42. Required equipment.

Materials

1 apple
sugar
tap water

Time Requirements

production of the juice: 20 minutes
production of the jelly: 25 minutes

Procedure

1. Cut an unpeeled apple into eight equal pieces, leaving the core intact. Place the pieces into the larger glass beaker, and just cover them with tap water.
2. Boil the mixture for ten minutes, stirring all the time. The pieces of apple must become mushy. Cool the coarse puree and press it through a muslin cloth into the plastic bowl.
3. Place the juice into the small glass beaker and weigh it (NOTE: weigh the empty glass beaker in advance.)
4. Add an equal amount of sugar and heat the juice again, stirring all the time.
5. After the juice has simmered for about five minutes, conduct a gelling test by observing the drops that fall from the wooden spoon. If the drops are thick and remain on the wooden spoon, you can allow the jelly to cool down. Divide the jelly and pour it into two glass beakers for this purpose. The gelling test can also be carried out by placing a little of the boiling juice onto a cold watch glass with a wooden spoon. If the juice gells on cooling, the boiling can be stopped.

NOTE: Do not boil the juice for longer than ten minutes, otherwise it will no longer gel.

Investigation O. Pectinase--An Enzyme that Decomposes Pectin (after Ch. Labahn-Lucius, 1990)

In this test, freshly pressed apple juice is replaced with equal parts of pure alcohol. The pectin in the juice forms an insoluble gel with the alcohol. Juice to which pectinase has been added does not produce gel, however, and also deposits substances that make the juice cloudy (figure 43).

Note on the blackboard the students' observation that the pectinase solution prevents gelling and causes a clarification of the juice. Use figure 44--a model of the pectinase effect--to help students realize that pectinase is an enzyme that decomposes pectin.

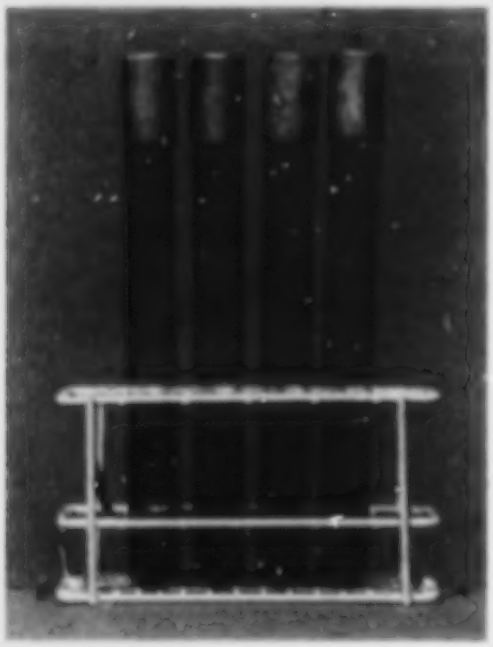

Figure 43. Alcohol test to determine the presence of pectin.

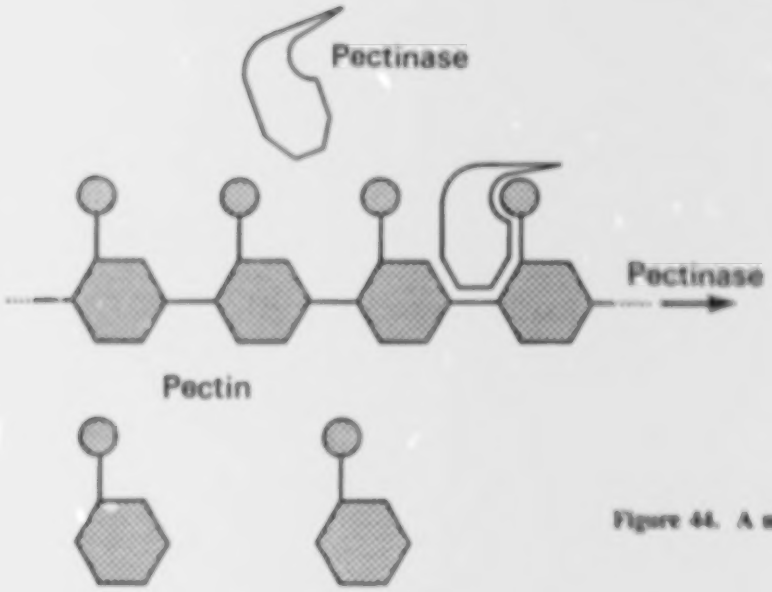

Figure 44. A model of the pectinase effect.

Prerequisite Biological Knowledge

the ecological significance of microbic decomposers
the effect of enzyme
structure of plant cells
the occurrence of pectin

Equipment

6 test tubes with bungs
3 pipettes (5 ml)
1 test-tube stand
2 pipetting aids
1 measuring cylinder
1 kitchen grater
1 glass beaker (50 ml)
2 bowls (plastic)
1 glass beaker (100 ml)
1 cotton nappy
1 glass rod

Materials

1 apple
5 ml 5 % pectinase solution
30 ml 96 % alcohol (denatured alcohol may be used)

Time Requirements

45 minutes

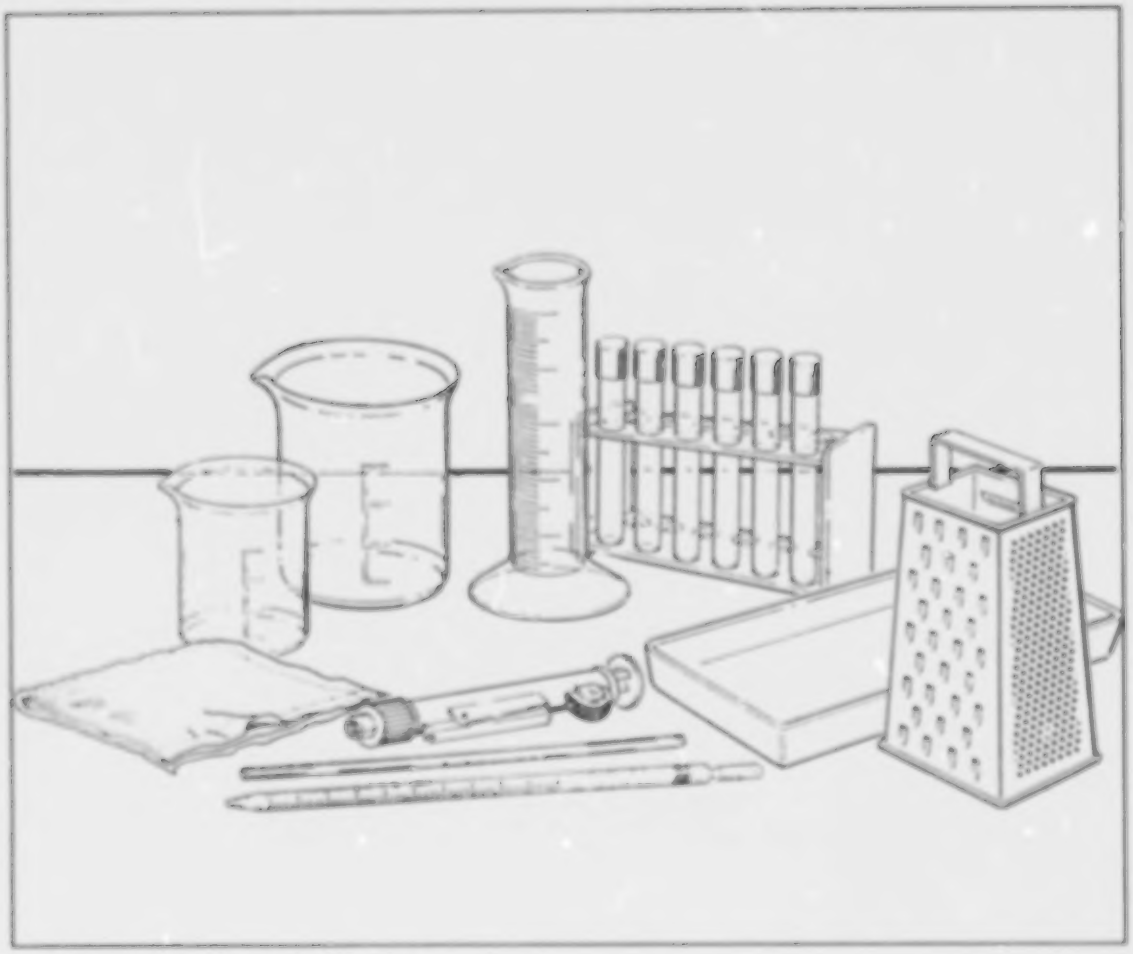

Figure 45. Required equipment.

Procedure

1. Grate the unpeeled apple into a plastic bowl. Squeeze the juice vigorously out of the puree over the second plastic bowl through a nappy folded double. Transfer the juice to a glass beaker. Note: a medium-sized apple such as a Granny Smith produces about 50 ml of juice.
2. Pour 10 ml of the juice and 2 ml of the pectinase solution into a glass beaker, shake the mixture. Position the glass beaker so that it will stand completely still. Observation: substances that make the juice cloudy are deposited.
3. Put 5 ml of the juice and 5 ml of alcohol into a test tube. Close the tube with a

bung, shake it carefully twice, and let it stand.

4. Add 3 ml of pectinase solution to the remaining juice (35 ml), stirring constantly. Start the stop watch. At three, six, nine (and possibly twelve) minutes, pipette 5 ml of the juice out of the glass beaker into a test tube, mix with 5 ml alcohol, seal, and rotate carefully twice. Place each of the test tubes as still as possible in the test-tube racks.
5. After each test tube has stood for at least five minutes, swirl it (carefully) to ascertain whether the flocculations remain as a clump of gel on the surface or whether they collect as loose components at the bottom of the test tube.

Investigation P. Industrial Use of Pectinase (after Ch. Labahn-Lucius, 1990)

In this investigation, the students determine the amount of juice produced from apple mash with and without the addition of pectinase. The fact that pectinase increases the juice yield raises the question: what is the significance of the use of pectinase for the fruit-juice industry? The teacher should bring various natural or concentrated fruit juices to class and inform the students about the production of juice. The class should discuss the reasons for using pectinase, for example, increased yield, the clarification of juice in response to customers' demands, and the reduction of transport costs. Include in the discussion trade terms such as "naturally unclarified," "clear," "concentrated," and "concentrate." The students should be informed that juice is still produced by the normal pressing technique. Conclude the teaching unit with a short role play in which manufacturers and customers discuss the reasons for and against the use of pectinase.

Reasons for the use of pectinase include increased yield, energy savings because the juice is easier to press, more economic methods of transport, and the ability to transport juice over longer distances.

Reasons against the use of pectinase include interference with the natural flavor and consistency of the juice, less native fruit is processed, inability of small cider companies to compete with producers of cheap juices.

Prerequisite Biological Knowledge

effect of enzymes
construction of plant cells
the occurrence of pectin

Equipment

2 tea strainers
2 funnels
1 kitchen grater
1 plastic bowl
2 glass beakers (100 ml)
2 glass rods
2 spoons
2 stands, sleeves, and stand clamps
2 measuring cylinders (50 ml)
1 set of scales

Materials

2 apples
10 ml pectinase solution (freshly made; 5%)
tap water

Time Requirements

25 minutes

Procedure

1. Grate both unpeeled apples over a plastic bowl, using a household grater.
2. Divide the apple mash equally between the two glass beakers (A and B), with the help of the scales.
3. Pipette 10 ml pectinase solution into glass beaker A , and 10 ml water into glass beaker B. Allow the glass beakers to stand as they are for ten minutes. Stir the mash at one-minute intervals, using glass rods.
4. In the meantime, attach the funnels to the stands, using clamps. Place a tea strainer into each funnel and place the measuring cylinders under the funnels. (Do not forget to stir the mash!)

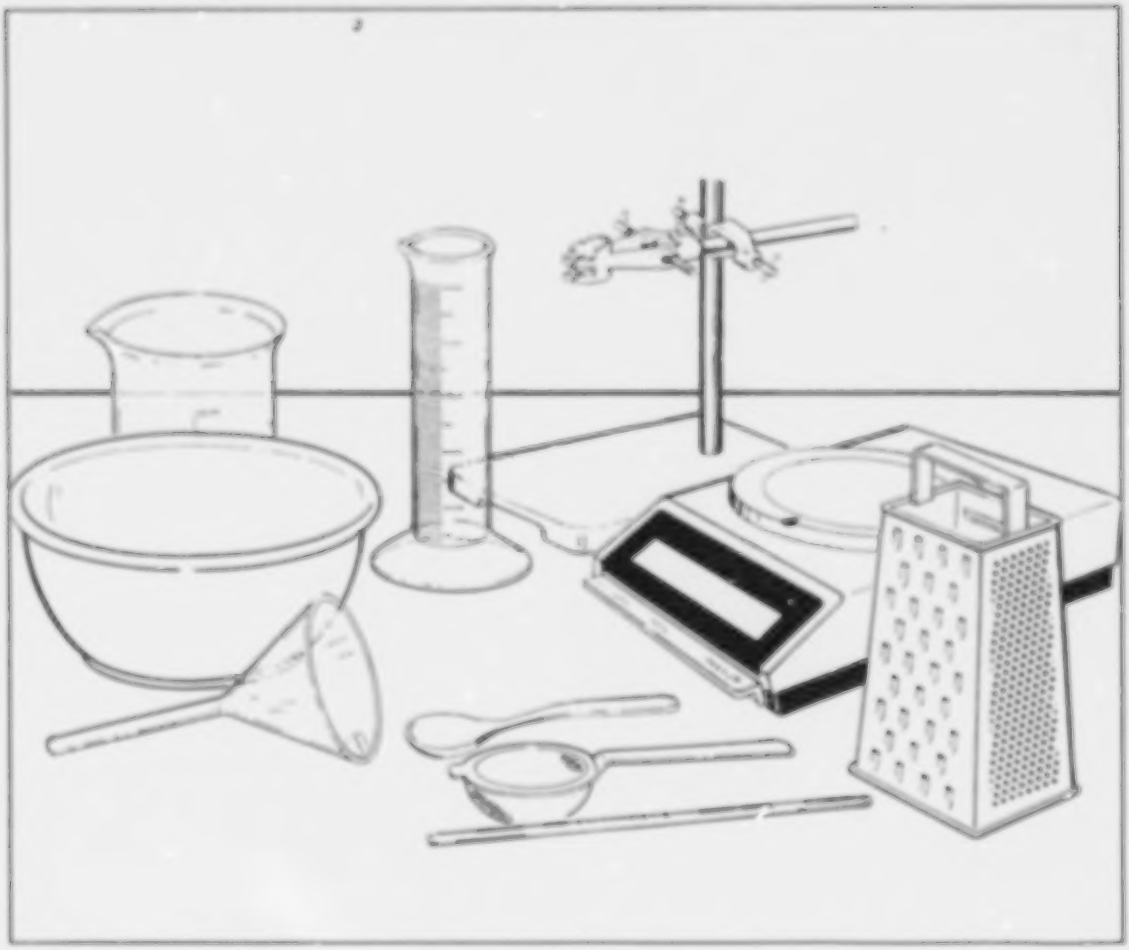

Figure 46. Required equipment.

5. After the time has elapsed, tip out of the glass beakers the apple mash A and B into tea strainers. You may need spoons to do this. The mash may not be pressed into the tea strainer.
6. After approximately five minutes, measure the quantity of juice in the cylinders.

Investigation Q. The Splitting of Lactose from Milk or Whey by Immobilized Lactase (after Ch. Labahn-Lucius & H. Plainer, 1990)

Enzymes that are not released to the environment but that are active in the inside of cells are formed by microorganisms in relatively small quantities. The industrial production of such enzymes, of which lactase is

one, is also quite tedious. The cells must first be broken open before enzymes of this kind can get into the culture medium from which they are produced. Dairies that use lactase for the treatmcnt of whey therefcre treat the expensive lactase with due care. It is immobilized before use, that is, it is bound to a vehicle. This allows several consecutive uses of the enzyme because it does not have to be thrown away with the waste products after it has been used the first time. (The opposite is true of amylase, which is used in washing powder; this enzyme is naturally active outside the cell.)

Immobilized lactase can be used as often as desired for school experiments. It can be preserved with isopropanol (see method) and kept for six months in the refrigerator.

Prerequisite Biological Knowledge

the ecological significance of microbic decomposers
the effect of enzymes
the composition of milk
the difference between lactose and glucose
pH-values
buffer

Equipment

1 filter tube (Duran, pore size 40 - 100 µ, 20 mm)
1 suction flask with rubber bung attachment
1 Woulfe's bottle
3 conical flasks, 500 ml
1 water-jet vacuum pump
1 measuring cylinder, 100 ml
1 glass beaker, 100 ml
2 pasteur pipettes
2 glass beakers, 50 ml
3 rubber caps for pasteur pipettes
1 conical flask, 100 ml
1 (10 ml) pipette with pipetting aid
3 conical flasks, 300 ml
1 stand with 2 clamps and 2 nuts

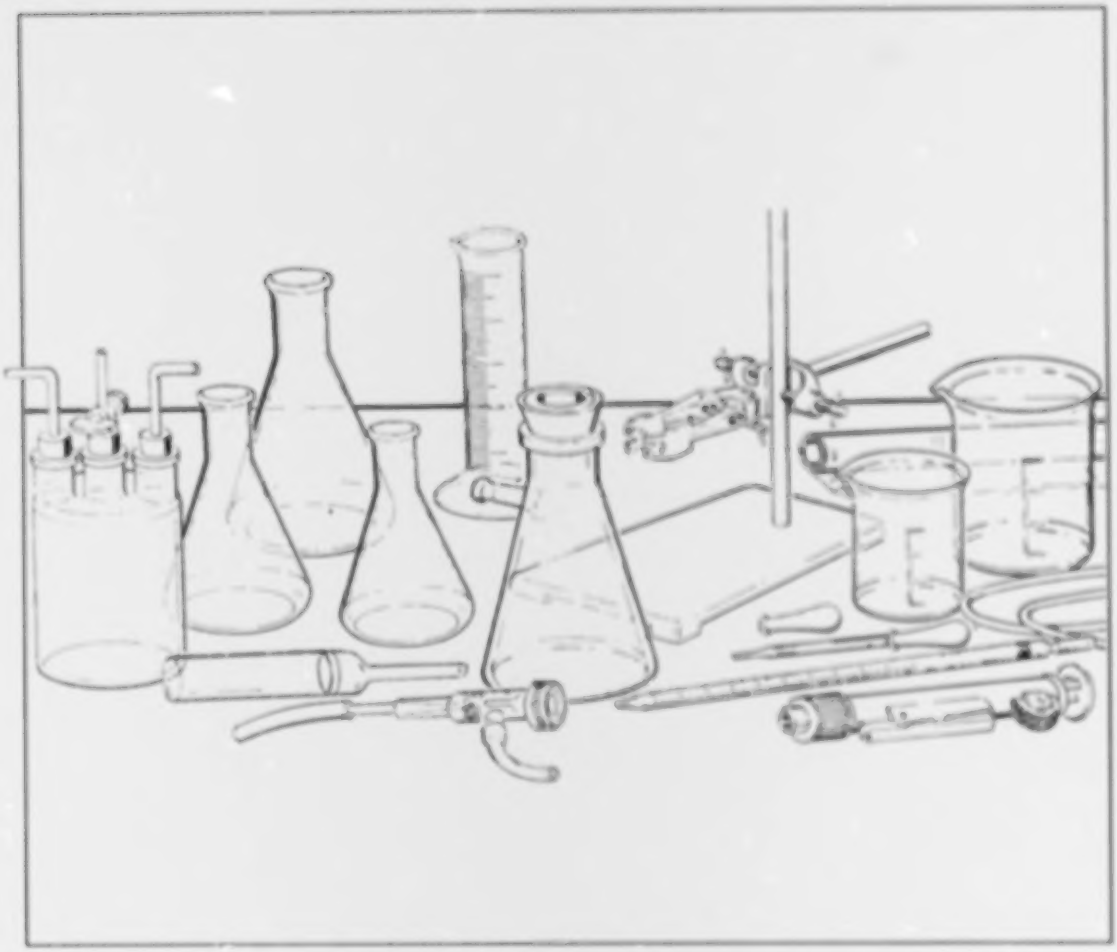

Figure 47. Required equipment.

Materials

1 piece of tubing
aluminum foil
approx. 5 ml isopropanol
lactase (can be ordered from the RÖHM PHARMA Ltd.)
sugar test strips (Diabur - Test 500, BOEHRINGER, 50 cost about 13 DM)
(BOEHRINGER, D-6800 Mannheim, FR Germany)
Eupergit C (can be ordered from the RÖHM PHARMA Ltd.)
(RÖHM PHARMA Ltd., Dr.-Otto-Röhm-Straße 2 - 4, D-6108 Weiterstadt, FR Germany)

whey (health-food shop) or skimmed UHT milk
20 ml 1 molar phosphate buffer reagent (see appendix)
approximately 150 ml 0.1 molar phosphate buffer reagent (see appendix)

Time Requirements

immobilization of the lactase: 10 minutes
waiting time: 2 days
splitting of lactose: 25 minutes

Procedure

1. Several days before conducting the investigation, immobilize the enzyme lactase as follows: dissolve 0.1 g lactase in 20 ml 1 molar phosphate buffer reagent in a (100 ml) glass beaker. Add 1 g eupergit C to this solution. Shake the suspension for a short while. Finally, seal the glass beaker with aluminum foil and allow it to stand for at least 2 days at approximately 20°C (room temperature). Shake the beaker now and again--about 2 to 3 times daily to facilitate the immobilization of lactase in eupergit.
2. After two days, place the suspension in the filter tube and place the tube onto the suction flask. Attach both to a stand and connect them to the Woulfe's bottle with a piece of rubber tubing attached to a water-jet vacuum pump.
3. Rinse the suspension in the filter tube with 40 ml 0.1 molar phosphate buffer reagent by rinsing it several times and removing the liquid by suction.
4. Finally, remove the suction flask. Place a 50 ml beaker under the glass beaker.
5. Add skimmed milk or whey drop by drop, using a pasteur pipette. There should be a surplus approximately one to three centimeters high above the eupergit. The milk products that drip out of the filter tube are caught in a glass beaker.
6. Test the milk products that have dripped through for glucose. The presence of glucose can be ascertained using glucose test strips that can be purchased from a supplier. The untreated milk or whey can be used as a control.
7. After the experiment has been completed, purify the immobilized enzyme with about 100 ml 0.1 molar phosphate buffer reagent until the filtrate is clear. In the last rinse, add 2 percent isopropanol by volume (preservation buffer reagent) to the phosphate buffer reagent to preserve the enzyme.
8. Seal the lower end of the filter tube with a rubber cap that is pushed over the end.
9. Add a preservation buffer reagent that contains isopropanol to the eupergit lactase compound until there is a surplus of one or two centimeters.

10. Close the filter tube with aluminum foil at the upper end and keep the tube in the refrigerator.

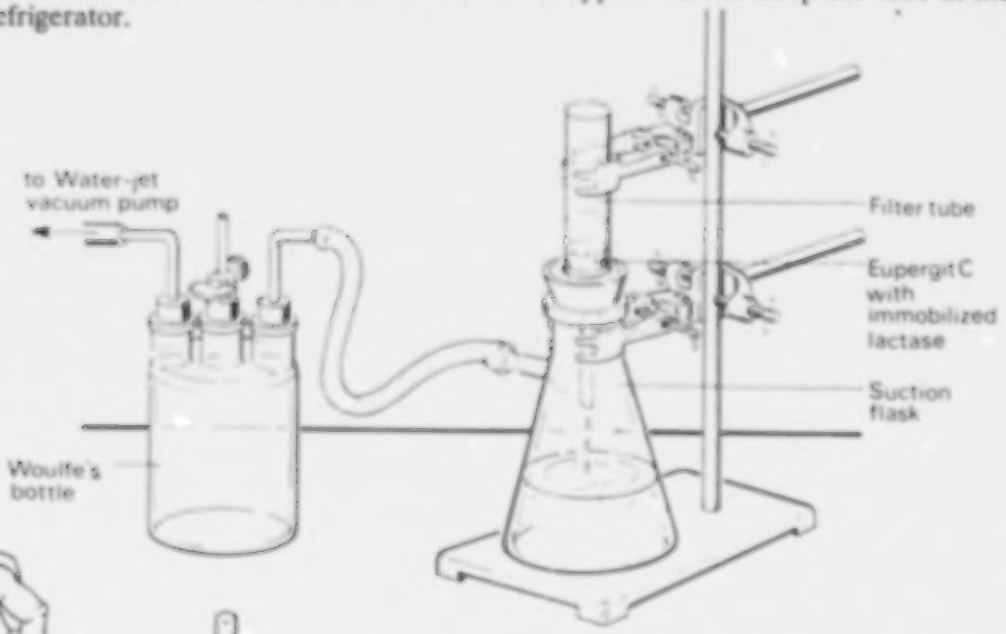

Figure 48. Washing the lactase that was immobilized using eupergit.

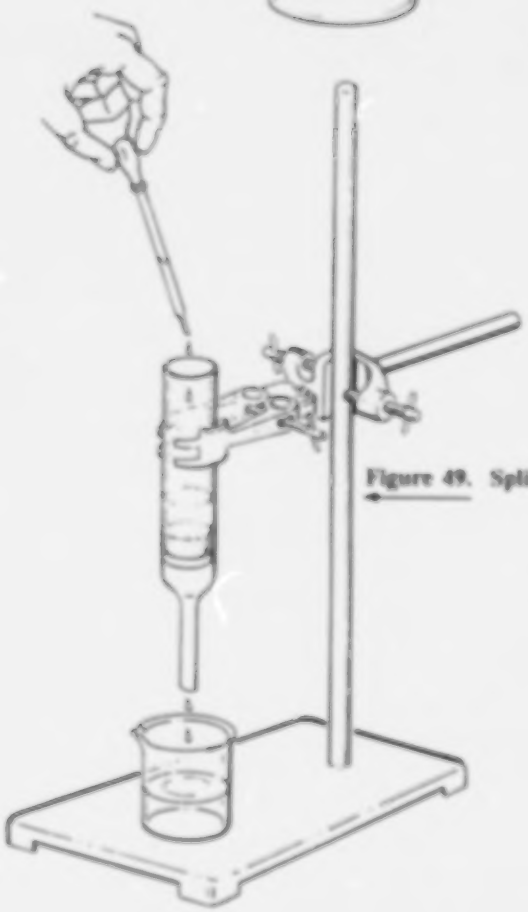

Figure 49. Splitting lactose.

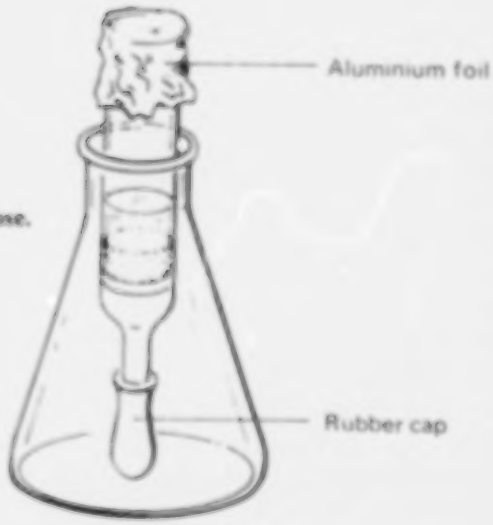

Figure 50. Storage of the immobilized lactase.

Palm oil is an important raw material in the production of margarine and cooking oil. It is, however, also used in the production of detergents in the washing powder and cosmetic industries. The Federal Republic of Germany imports approximately 400,000 tons of palm oil (and coconut oil) per year from Malaysia and the Philippines because the fats produced in Germany (sunflower oil, rape oil) do not possess the necessary characteristics (German Bundestag, 1987, pg. 50). The young oil palms are produced in vitro by Unilever and exported to Malaysia and the Philippines. This is an economic advantage for the farmers there. The young plants are bred from the tissue of a tree whose yield is particularly high so that it is expected that they will also produce good yields. Oil palms grown from seed sometimes yield less oil because of their biological variability; this only becomes apparent after approximately 10 years. When selecting plants from which tissue is removed, other important characteristics are also considered, for example, resistance to illness and acclimatization to poor soil. Fruits should also be easy to harvest (see Teasdale, 1987 and Ingleby, et al., 1987).

The cultivation of plant cells, tissues, or organs on artificial culture medium (*in vitro* culture) plays an increasingly important role both in the cultivation of plants and in the production of plants (see Chapter 2). This procedure is representative of a modern form of vegetative reproduction in which numerous genetically identical individuals are asexually produced from a parent plant (clones). We can produce clones like these in the usual way, using cuttings from shoots and leaves. In the case of cell or tissue cultures, a leaf or a shoot is cut into suitable parts that are then sterilized and grown either in a liquid or solid culture medium. The cells and tissues must be supplied with all the substances that plants require for growth (mineral salts, sugar, amino acids, vitamins, plant hormones). Sterile working conditions are essential, otherwise the plant material is quickly overcome by microorganisms. After a while, new shoots develop from the tissue, and the shoots can then be cultivated until the required amount has been produced. They must then be acclimatized to normal growth condition. *In vitro* reproduction is relatively work intensive (sterile working conditions, suitable nutrients) and is therefore carried out by specialized firms (tissue culture laboratories) that supply nurseries and forestry companies. Because the process requires a relatively high investment, *in vitro* cultures are only viable in the case of plants that are important from an economic point of view, more precisely for those that are difficult to produce from cuttings or exclusively from seed, those that produce only a limited number of seedlings, or those whose yield is reduced if they are grown from seed, because of genetic variation.

This modern form of vegetative reproduction has been widely accepted above all in the area of ornamental plants, but also in the cultivation of fruit trees and in forestry. The number of plants produced *in vitro* in Holland increased by 475% between 1980 (7.43 million) and 1986 (42.775 million) (Preil, 1987).

Experiments on the *in vitro* culture of plants can be carried out without any problem in schools. The following section describes an experiment on the reproduction of usambara violets. Economic calculations make the advantages or disadvantages of *in vitro* cultures versus seedlings clear; a calculation of this kind is carried out for the gerbera plant (after G. Hebenstreit of the IPN).

Investigation R. In Vitro Culture in the Ornamental Usambara Violet (*Saintpaulia ionantha*)

Numerous shoots develop from pieces of shoot or leaf of the usambara violet after 2 - 4 weeks if the pieces are placed onto a medium containing cytokinin (approximately 50 from a piece of leaf 0.5 cm^2). If the shoots are then transferred to a medium that does not contain hormones, it produces roots after about one week. The small plants can be cultivated further in plant pots. All of them produce flowers of the same color and otherwise possess similar characteristics. Here the students experience the conspicuous production of clones. The work must be carried out in sterile conditions or other microorganisms might be produced that would overrun the pieces of plant tissue in a very short time. A "sterile tunnel" (developed by Uta Nellen of the IPN after an idea by J. Carter [1986]) can be used for this purpose. Its construction is described below, together with U. Nellen's experiment on usambara violets.

R1. Making a Sterile Tunnel for *In Vitro* Cultures in Schools (after U. Nellen, 1988)

Prerequisite Biological Knowledge

No special knowledge is required.

Equipment

blowtorch (up to 600°C) with jet (usually suitable as a paint stripper)
2 screw clamps
1 wooden lath 1 cm x 1 cm x 50 cm

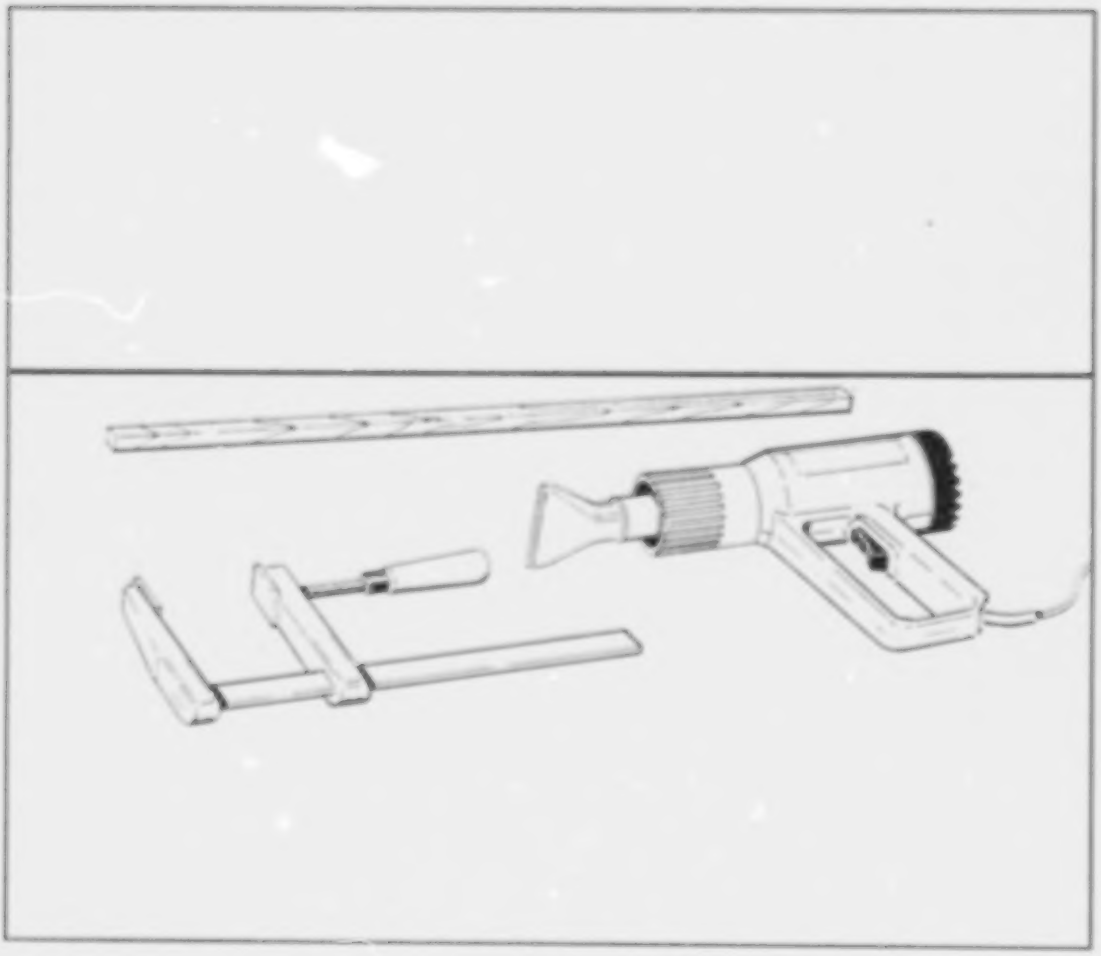

Figure 51. Required equipment.

Materials

plexiglass 30 x 45 cm; thickness: 3 mm

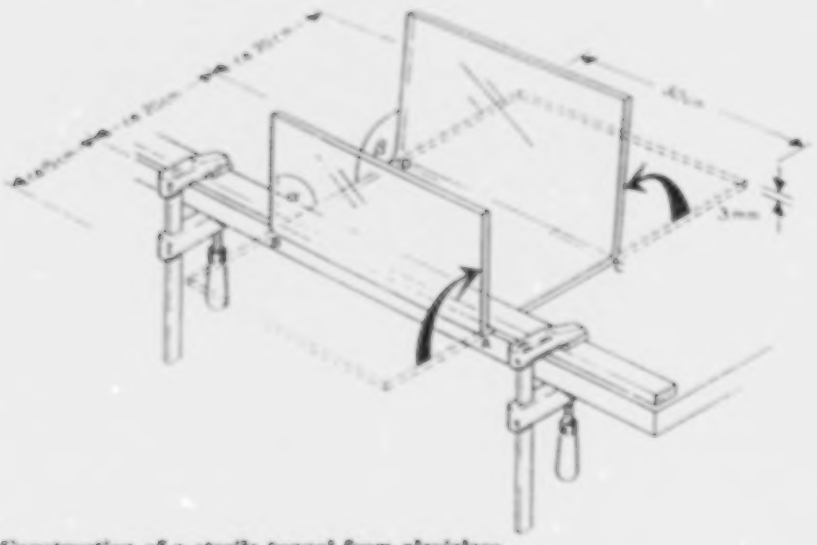

Figure 52. Construction of a sterile tunnel from plexiglass.

Procedure

1. File down the sharp edges of the plexiglass plate. Mark points A, B and C, D. Place the piece of plexiglass onto a wooden table (which is not coated with plastic) so that line A-B is exactly on the edge of the table. Place the wooden lath onto the plexiglass exactly on the edge of the table; secure the lath on both sides with screw clamps so that the plexiglass is between them. Heat the A-B line with the blowtorch until the plexiglass softens (approximately 1 minute at approximately 600°C); bend the plexiglass that juts out beyond the A-B line upwards at the desired angle. Hold the plexiglass until it cools.

2. Bend the plexiglass along the C-D line. To achieve the desk form of the tunnel, the and angles should be 90° and 110°, respectively. Several sterile tunnels can be piled on top of one another.

R2. <u>Production of Usambara Violets from Pieces of Leaf (from Nellen, 1988)</u>

Before tissue cultures are prepared, prepare petri dishes, using MS medium as a culture medium (see appendix). Before this is done, add BAP strain solution in the ratio of 0.5 ml per liter of culture medium. (BAP: 6-benzylaminopurine, a cytokinin).

Pour the medium into sterile, disposable petri dishes (diameter 9 cm) while it is still hot (> 50°C). One liter is sufficient for approximately 35 dishes. Stack the dishes to avoid the formation of condensation in the lids of the petri dishes and to protect the surface of the table. Label five empty petri dishes with the date and type of medium and pile the dishes on top of one another. Lift up the whole pile with the lid of the lowest dish, pour the medium into the lowest dish, cover it with the lid and the rest of the pile. Lift the lid of the next dish, together with the rest of the pile; place the medium into the next dish, and so on. After approximately one week, place pieces of the leaf onto the sterile culture media. Plates that should be kept for longer periods of time are packed in cling film or plastic bags to prevent their drying out and to protect them from contamination.

Prerequisite Biological Knowledge

vegetative reproduction of plants
nutrition of plants
plant hormones

Equipment

1 bent pair of tweezers (sterile)
1 scalpel (sterile)
1 kitchen timer
1 container for decanting liquids
4 glass beakers, 200 ml, sealed by a glass petri dish (sterile)
1 sterile tunnel
1 bunsen burner
adhesive tape
transparent freezer bags
wooden sticks ca. 10 cm

Materials

MS medium (see appendix)
BAP strain solution (see appendix)
70% alcohol (denatured alcohol may be used), approximately 100 ml

96% alcohol (denatured alcohol may be used), approximately 100 ml
dilute domestos solution (disinfectant), 20%, approximately 100 ml
sterile tap water, approximately 100 ml

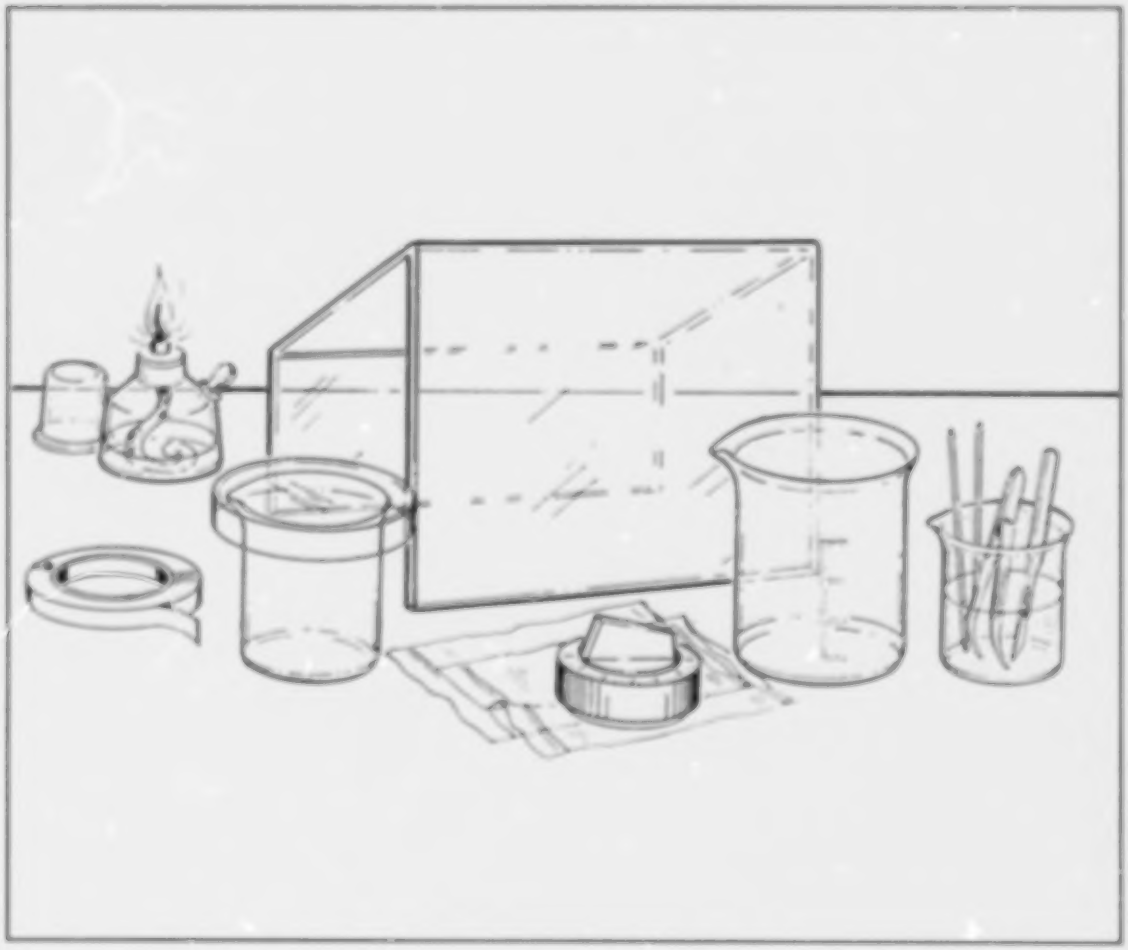

Figure 53. Required equipment.

Procedure

1. Rinse a leaf of an usambara violet in 70 % alcohol in a sterile glass beaker for about 1 minute. The glass lid must only be opened for as short a time as possible and must be replaced immediately! Carefully decant the alcohol without removing the lid so that the objects do not slip out.

2. Add dilute domestos solution and shake the glass beaker. Sterilization time: 1 - 2 minutes. Decant the solution as in 1.
3. Rinse the leaf in sterile tap water three times per 5 - 10 minutes; shake slightly with the lid closed. Carefully decant the last water used for rinsing.

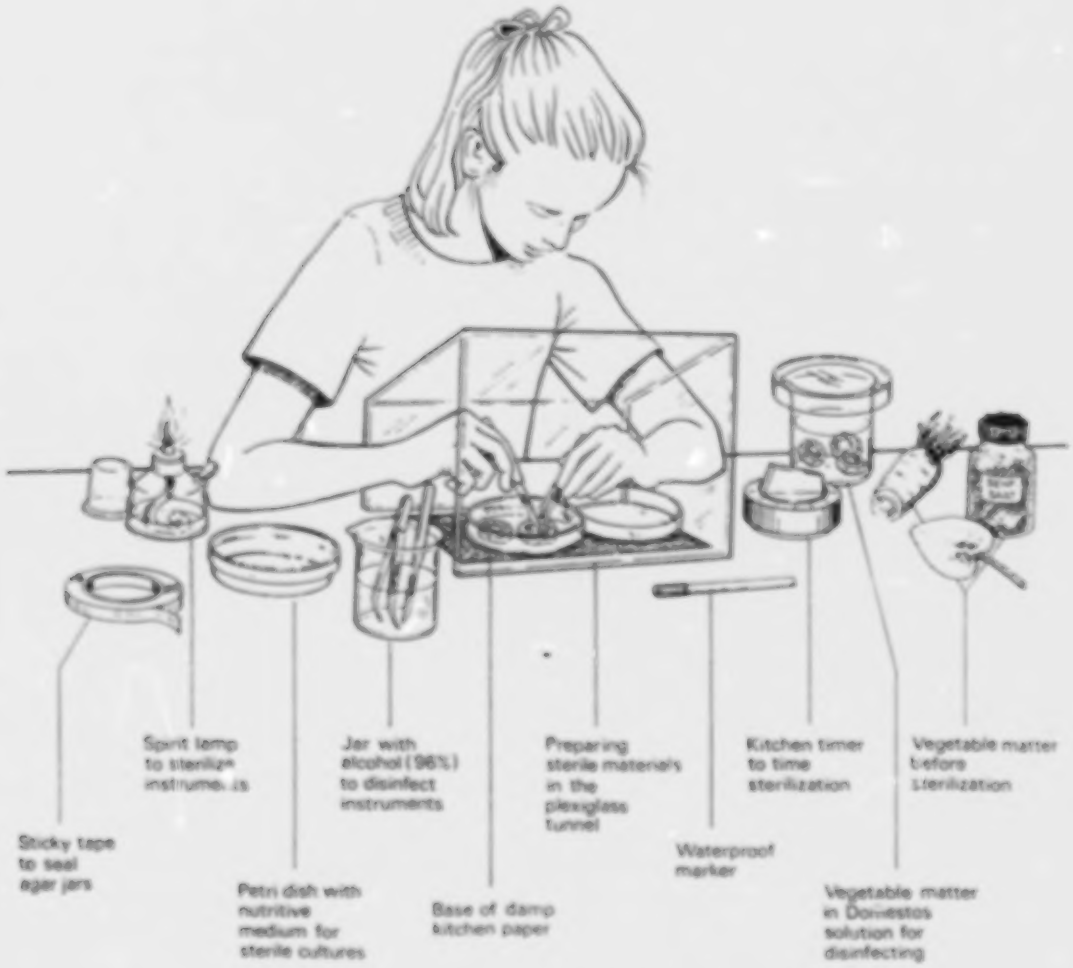
Sticky tape to seal agar jars
Spirit lamp to sterilize instruments
Petri dish with nutritive medium for sterile cultures
Jar with alcohol (96%) to disinfect instruments
Base of damp kitchen paper
Preparing sterile materials in the plexiglass tunnel
Waterproof marker
Kitchen timer to time sterilization
Vegetable matter in Domestos solution for disinfecting
Vegetable matter before sterilization

Figure 54. Experiment for the cultivation of a tissue culture in a sterile tunnel.

4. Take the leaf out and place it onto an empty sterile petri dish. (The tweezers used for this step are sterilized in 96% alcohol before use.)
5. Cut away the tissue at the edge of the leaf that was damaged during the process of sterilization. Also cut away the larger vascular tissue. (Use the scalpel, sterilized in 96% alcohol.)
6. Cut the leaf into strips about 5 mm wide and 1 cm long.
7. Gently press the strips of leaf onto the culture medium that contains cytokinin. Close the petri dish and seal it with adhesive tape. Place the petri dish into a well-lighted place for about 2 - 4 weeks (room temperature).
8. When shoots form, transfer them to culture medium without cytokinin so that they form roots.
9. After approximately two weeks, as soon as small roots have been formed, transfer the plants to plant pots. These in turn are placed into freezer bags that are tied at the top. Place a small wooden stick into the soil.

Explanations and Suggestions

Step 1. The rinsing of the leaf increases the wetness of the surface.

Step 5. Microorganisms that were not killed during the sterilization of the surface may be present in the vascular tissue.

Step 9. The freezer bag is waterproof, but porous to air. The wooden stick prevents the plastic of the bag from pressing on the plants.

Investigation S. **The Cultivation of Gerbera: More Economical by *In Vitro* Culture (after H. Bayrhuber, 1990)**

How much profit does a gardener make if she plants 1,000 plants that have been produced *in vitro*? How much profit does she make if she sows seedlings? She must buy both the young plants that have been produced *in vitro*, and the seed. Because the gerbera seeds do not germinate very well, she must buy an average of 1,430 seeds (at DM 0.64 each) if she wishes to grow 1,000 young plants. While they are being grown, there are losses, and a number of young plants do not blossom, so the gardener is only able to sell 700 of the 1,000 young plants grown from seed. The losses made from the young plants grown *in vitro* were less; 950 of 1,000 young plants could finally be sold as pot plants.

Table 1 is a comparison of the cost calculations. Overhead refers to the financial cost of the required area in the greenhouse multiplied by the

number of days during which this area is occupied by plants (m^2 x days = day square meters = Dm^2). A gardener requires, for example, 0.35 m^2 x 35 days = 12.25 day square meters. One day square meter costs DM 0.15, that is, the overhead is DM 1.84 in this case. The plants that have been produced *in vitro* can be compared to seedlings that are seven weeks old. The former come into flower within one to three weeks, while eight weeks elapse between the flowering of the first and last plants produced from seed. Therefore, the overhead for the plants produced *in vitro* is considerably less.

Collateral refers to the difference between the proceeds and special costs. This covers the overhead. The difference between the collateral and the overhead is profit. In the case of 1,000 potted gerbera plants produced *in vitro*, profit is about DM 600 higher than if the plants were sown from seed. These data help convince students of the success of *in vitro* culture in the production of gerbera potted plants (after Hebenstreit, 1986).

Prerequisite Biological Knowledge

vegetative and sexual reproduction of plants

Potted Gerbera from young plants produced *in vitro*. Cost calculation for 1,000 potted plants.

Income		Expenses		Assets	
950 plants (at DM 5.00 each)	DM 4,750.00				
		Young plants (DM 2.20 - 2.60 each)	DM 2,400.00		
		Substrates, plant pots	DM 150.00		
		Fertilizer, plant protection	DM 150.00		
		Heating costs (604 litres oil)	DM 181.20		
		Wages (13.5 working hours DM 15 per hour)	DM 202.50		
		Preparation for sale (950 plants at DM 0.15 each)	DM 142.50		
		Variable special costs in total	DM 3,226.20		
		Overhead (3,525 Dm^2 at DM 0.15 each)	DM 528.75		
				Collateral	DM 1,523.80

Total Profit = DM 4,750.00 - DM 3,754.95 = DM 995.05

Potted Gerbera from Seedlings. Cost calculation for 1,000 potted plants.

Income		Expenses		Assets	
700 plants (at DM 5.00 each)	DM 3,500.60				
		Seeds (1,430 seeds at DM 600 per 1,000)	DM 915.20		
		Substrates, plant pots	DM 150.00		
		Fertilizer, plant protection	DM 200.00		
		Heating costs (958 litres oil)	DM 287.40		
		Wages (18 working hours at DM 15)	DM 270.00		
		Preparation for Sale (700 plants at DM 15 each)	DM 105.00		
		Variable special costs in total	DM 1,927.60		
		Overhead (8,452 Dm^2 at DM 0.15 each)	DM 1,267.80		
				Collateral	DM 1,572.40

Total Profit = DM 3,500 - DM 3,195.40 = DM 304.60

Table 1. The cost efficiency of various cultures of potted gerbera (figures after U. Schmoldt, Gartenbaubetrieb, Funfhausen; oil price from autumn 1988).

DNA Technology

"Monsanto GmbH Deutschland has petitioned the Federal Health Agency to permit treatment of dairy cows with a growth hormone. The hormone is produced with the help of bacteria into which the gene for the growth hormone has been introduced.

The hormone regulates the animals' growth and promotes milk production in adult animals. Regular injections of the hormone can increase a cow's milk yield by 7 to 14 percent. Administration of the hormone is possible only on relatively large and specialized dairy farms, because the animals, exposed to considerable physiological pressure, require intensive care, and often particular medical attention. Opponents of the petition fear, among other things, that an increase in the yield of large-scale dairy concerns could result in the enforced closure of small and medium-sized dairy farms. The composition of the milk is also expected to change under high-yield conditions, impairing the quality of the dairy products" (Bayrhuber 1990).

Grade ten students can readily understand passages of this kind, and they also can be taught how to evaluate the contents provided they have a basic understanding of the concept of a gene (see Chapter 2): "a gene is a piece of DNA that controls the production of a peptide in a cell." The students must also, of course, be in a position to judge human actions, such as the use of genetic technology in agriculture, by applying moral criteria (see Chapter 3). The next two investigations explore genetic technology (see Chapter 2). The final section of this chapter includes three case studies that require the application of genetic technology in humans.

1. ***Teaching Genetic Technology Through Experiments.***

This is problematic in two respects. A number of countries, including the Federal Republic of Germany, prohibit experiments in genetic technology in classrooms. One can overcome this difficulty by using bacterial investigations where natural exchange of genetic material occurs; these investigations can then serve as a model for induced gene transfer. The second problem is whether it is worth doing such experiments in the tenth grade at all, because it is doubtful that they can be interpreted adequately. We can examine this issue using the illustration of bacterial conjugation.

Grade ten students can be told that in conjugation a plasma brige forms between two parallel bacteria, through which the genetic material passes from one cell (donor cell) to the other (recipient cell). The concept "genetic material" can be specified in more detail as a sub-strand or part of a sub-strand in the DNA of the donor cell. The students can also be told that parts of the transferred genetic material are incorporated into the chromosome of the recipient cell, where they become active, while the rest, together with the displaced material of the recipient chromosome, are broken down in the cytoplasm. Finally, the students can learn that the genetic material missing in the donor cell is

synthesized in that cell. Conjugation is distinguished from transformation (uptake of free DNA by the bacterial cell) and transduction (transfer of genetic material via bacteriophages) at the same general level.

Further details of conjugation are unnecessary and inappropriate at this level. Whether the experiments are worth doing depends upon the educational goals determined by the teacher and the science program in general. Which goals could apply here? The conjugation experiment is intended to help the students learn that genetic material is transmitted among bacteria under natural conditions. They should realize that the spread of recombinant DNA from experiments in genetic technology is, in principle, possible in the same way. This understanding can lead to discussions of the safety of genetic technology, where students should recognize the fundamental difference between conjugative gene transfer in nature and intentional genetic manipulation by humans: intervention by means of genetic technology has moral relevance as a human action. With reference to standards governing human actions, such intervention cannot be justified simply by the fact that transmission of genetic material occurs in nature. The investigations of conjugation promote the major educational objectives, but teachers may prefer to postpone such investigations until later grades.

Investigation T. Isolating DNA from Sweetbread (from H. Bayrhuber, Ch. Gliesche, E. R. Lucius, 1990)

Sweetbread is the thymus gland, usually of calves, the cells of which posses relatively large nuclei from which large amounts of DNA can be obtained. Sweetbread can be bought from a butcher; fresh and deep-frozen samples produce comparable results.

Prerequisite Biological Knowledge

isolation of DNA
basic idea of the construction of DNA
function of the thymus gland and position of the gland in the body

Equipment

1 glass beaker, narrow, 100 ml
1 mortar
1 pestle
1 pair of scissors

1 scalpel
1 glass rod
1 pair of tweezers
surgical gaure

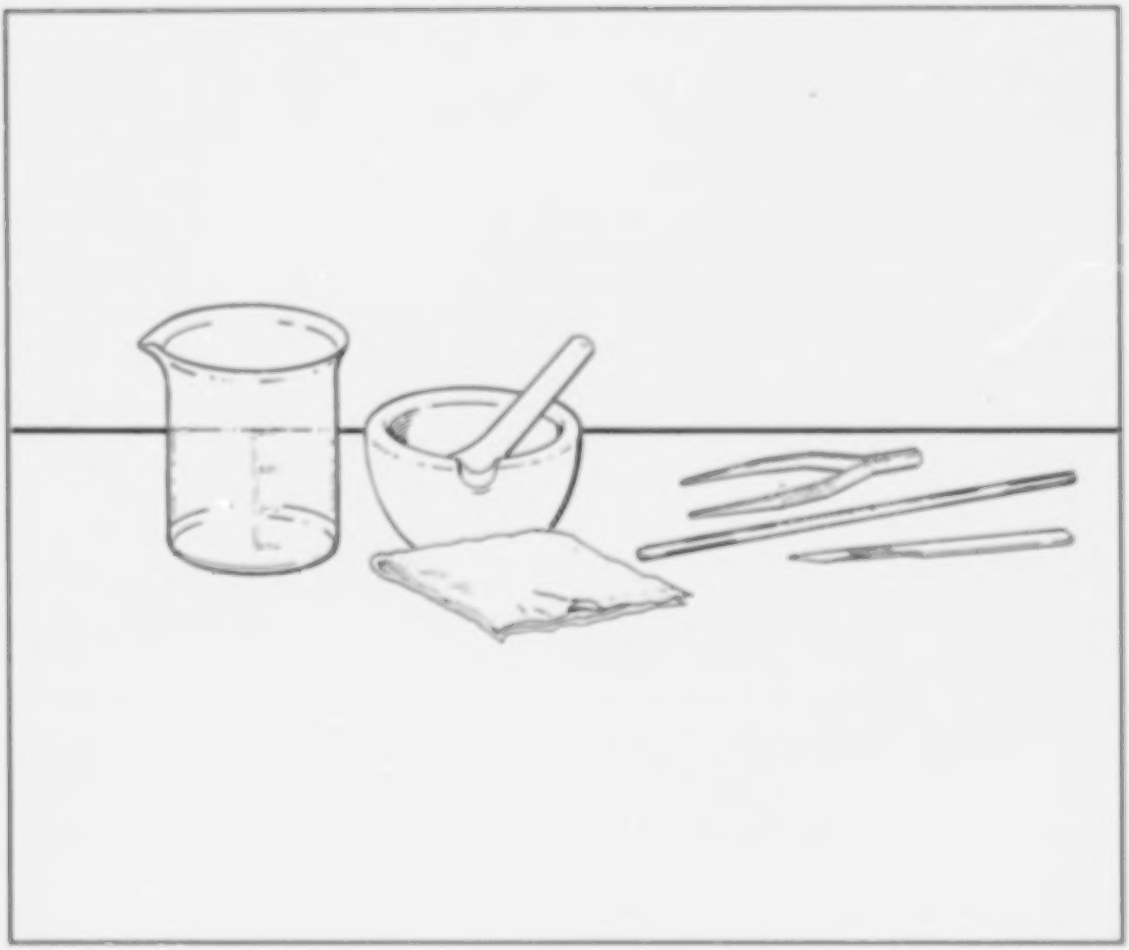

Figure 55. Required equipment.

Materials

5 -10 g sweetbread
abrasive sand
tap water

washing-up liquid (containing tensides)
ethanol 96% (or methylated spirit)

Procedure

1. To produce a thymus suspension, cut up 5 - 10 sweetbreads in a mortar using a pair of scissors or a scalpel.
2. Add sand to the tissue in the mortar and grind with the pestle; then add approximately 20 ml tap water and continue grinding until the water is completely clouded.
3. Filter the suspension through two layers of surgical gauze.
4. To liberate DNA, add approximately 5 - 10 drops of washing-up liquid to the cell suspension and shake thoroughly.
5. To obtain DNA, cover the clear, viscous suspension with approximately one and a half times the amount of cold ethanol solution in a narrow glass beaker.
6. The DNA precipitating as whitish strands can be removed with a glass rod. Rotate the rod slowly and carefully dip it up and down occasionally to mix the suspension and the alcohol. This will precipitate a relatively large amount of nucleic acid. If the strands slip from the rod, they can be fished out of the beaker using a tweezers.

Explanations and Suggestions

Step 2. The water is clouded by cells and cell detritus that have been abraded and are suspended in the water.

Step 3. The suspension is filtered to separate off the sand and the tissue detritus.

Step 4. Each tensided molecule of the washing-up liquid has a hydrophilic and a lipophilic end. When they are taken up, the field of charge of molecules of the lipoprotein membrane is shifted, and hydrolysis occurs. In this way, for example, the membranes of the cell and nucleus are destroyed and histone molecules split off from DNA strands. After the addition of washing-up liquid, the suspension becomes clear and viscous. The viscosity is caused by liberated DNA molecules.

Investigation U. Conjugation in Bacteria (from Ch. Gliesche, 1990)

The strain *Escherichia coli* GY767 (DSM-No. 1562) is streptomycin sensitive. The F plasmid is integrated into its chromosomal DNA, and it is able to transfer chromosomal genetic information to strains of *E. coli* that have no F plasmid. In this experiment, the recipient is *E. coli* AB1157 (DSM-No. 1563). This

streptomycin-resistant strain is characterized by a large number of mutations and can no longer produce for itself the amino acids proline, leucine, arginine, threonine, or histidine, or the vitamin thiamine, which it requires to survive. These substances must therefore be present in the culture medium if growth is to occur. In the following experiment, donor cells (*E. coli* GY767, DSM-No. 1562) and recipient cells (*E. coli* AB1157, DSM-No. 1563) are mixed in a ratio of 1:9. Within two hours the chromosomal genes for the biosynthesis of proline, threonine, and leucine are transferred from the donor to the recipient. Recipient cells are distinguished from the donor cells by their resistance to streptomycin. After transfer of the genes for biosynthesis of the amino acids proline, leucine, and threonine, recombinant recipient cells (now also called transconjugants) grow on minimal agar that contains arginine, histidine, thiamine, and streptomycin, but not proline, leucine, or threonine. Neither the donor, with its streptomycin sensitivity, nor the unchanged recipient, which would need the missing nutrients prolin, leucin, and threonin, can grow on this minimal agar (figure 56).

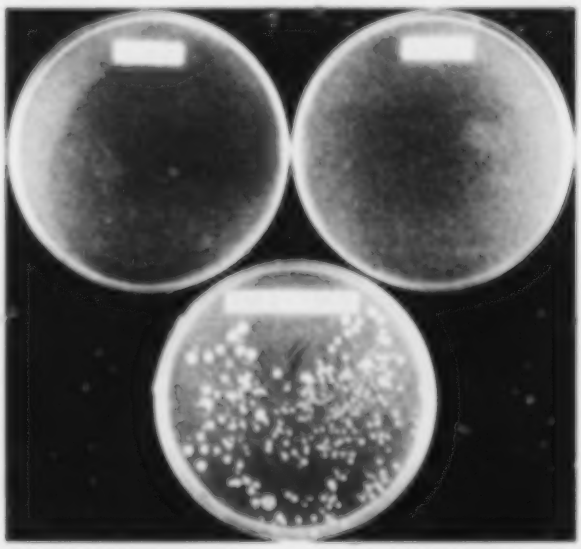

Figure 56. Conjugation between *E. coli* GY767 (DSM-No. 1562), a wild strain with chromosomally integrated F-plasmid, and *E. coli* AB1157 (DSM-No. 1563), a multiple auxotrophic mutant. Neither strain can grow on streptomycin-containing minimal agar. Transconjugants of *E. coli* AB1157 (DSM-No. 1563) develop on this medium only after conjugative transfer of genetic information.

Prerequisite Biological Knowledge

conjugation in bacteria
the concept of a gene
basics of bacterial genetics
the sexuality of bacteria
the effect of antibiotics
resistance
mutation
amino acids

Equipment

incubator
1 glass beaker, 250 ml
1 conical flask, 250 ml
3 petri dishes
3 test tubes with aluminum caps or cotton wool bungs
1 pipette, 5 ml, sterile
4 pipettes, 1 ml, sterile
pipette aids
1 Drigalski spatula
1 bunsen burner

Materials

overnight culture of *E. coli* GY767 (DSM-No. 1562)
overnight culture of *E. coli* AB1157 (DSM-No. 1563)
nutrient broth medium for the overnight culture (see appendix)
minimal agar medium (see appendix)
streptomycin, 0.02 g
distilled water
alcohol, 70%

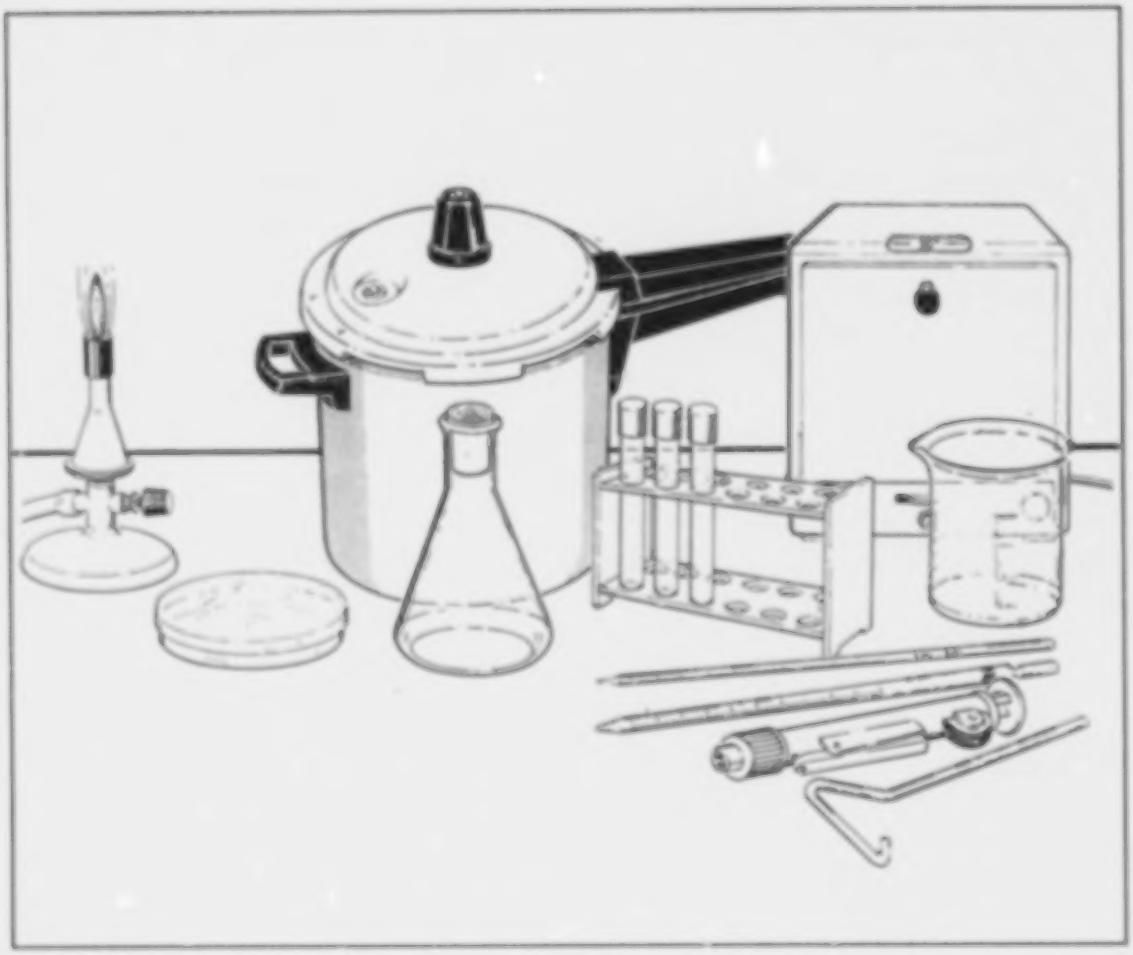

Figure 57. Required equipment.

Procedure

1. Using the Erlenmeyer retort, prepare minimal agar with 100 ml distilled water (see appendix) and adjust to a pH value of 7.2 using 1 n NaOH. Seal the retort with aluminum foil and sterilize, then allow to cool to 55°C. Add 0.02 g streptomycin and firmly shake the retort to dissolve. Pour into three agar dishes.
2. Prepare 5 ml each of overnight culture of *E.coli* GY767 (DSM-No. 1562) and *E. coli* AB1157 (DSM-No. 1563) (see appendix).
3. Mix 0.3 ml *E. coli* GY767 (DSM-No. 1562) overnight culture and 2.7 ml *E. coli* AB1157 (DSM-No. 1563) overnight culture in a sterilized test tube and incubate at

37°C for two hours.

4. Spread 0.1 ml of this mixture onto a minimal agar dish using the Drigalski spatula (sterilized with 70% alcohol and flamed in the bunsen burner). Prepare two control dishes with 0.1 ml of *E. coli* GY767 (DSM-No. 1562) and *E. coli* AB1157 (DSM-No. 1563) overnight culture respectively. Incubate all three dishes at 37°C for 72 hours.

Explanations and Suggestions

E. coli GY767 (DSM-No. 1562) shows no signs of growth on the nutritive medium, as it is streptomycin-sensitive. Nor can *E. coli* GY767 (DSM-No. 1562) grow on this medium, because the amino acids required by this strain--proline, leucine, and threonine--are not present. Cells of *E. coli* AB1157 (DSM-No. 1563) can grow on this medium only when they have received from the donor the genes for biosynthesis of these amino acids. During the experiment, genes for arginine, histidine, and thiamine biosynthesis are also transferred, but not detected.

Biotechnology and Ethics: Selected Case Studies

Two questions are of particular concern with regard to the integration of valuing and reasoning processes (see Chapter 3):

a. Does human dignity remain inviolate, or do human beings become objects?

b. Does biotechnology enhance or detract from the well being of humanity and nature?

Human dignity and well being are basic cultural values that are used in ethical reasoning as criteria for justification of human action (see Chapter 3). The specific definitions of dignity and well being may differ from culture to culture, and they may be complemented by other criteria. The values of dignity and well being do, however, allow judgments that are independent of cultural differences.

In the ethical assessment of biotechnology, however, the application of those criteria may well lead to an irreconcilable opposition of conclusions, so that teachers and students who embark on such a discussion in biology lessons are bound to find themselves in a perplexing situation. It is most certainly not the usual goal of biology teachers to present their students with irreconcilable conflicts or to lead them into dilemmas. Should teachers, therefore, refrain from introducing such problems for discussion? The fact remains that students are confronted with contradictory arguments outside school, and in these situations they do not as a rule encounter any reference to ultimate ethical values. The necessary basis for ethical reflection is thus lacking, and biology education should attempt to provide some of the basic skills.

Familiarization with naturalistic and personalistic arguments (see below) could make students more responsive to questions of whether human dignity and well being are more important to this society than are exploitation of fellow humans, and economic advantage. Students may develop a critical attitude quite spontaneously in the context of ethical justification. Perhaps the person who has learned and accepted that there is not one, wholly-valid basis of justification for standards of action will tend less towards rigorous rejection of those who act differently. Perhaps instruction about ethics will increase tolerance for divergent viewpoints.

We must seriously consider whether teachers should always have clear-cut, unambiguous solutions to offer their students. Clearly, teachers should not preempt the students' own experience by making the decisions for them. No doubt the students will want to know what choice the teacher would finally make if he or she were in a situation where a decision were called for, so the teacher may not be able to avoid giving a personal opinion. The teacher's position and authority, however, may easily persuade students

prematurely, so it is wise to withhold personal opinions until the students have analyzed the relevant information and come to their own conclusions. It is important to consider the age at which students can understand and discuss irresolvable conflicts of the kind described above, and at what age they can analyze such issues on the basis of ethical ideals such as well being and human dignity. This is, for example, obviously impossible for students at what Kohlberg (1976) defines as stage 2 of moral development. Furthermore, it is unclear how many students will indeed be guided by these principles in the future (stages 5 and 6 according to Kohlberg, 1976). It seems impossible for the teacher to influence the later decisions of students directly and ultimately; all he or she likely can hope for is to make the students familiar with the possibilities of an ultimate ethical justification.

Biology teachers can do no more for their students than to help systemize the choices that would be open to them in such situations. They can make it clear to the students that where difficult decisions of conscience are required, the limits of rational analysis soon become apparent, and the importance of values becomes obvious. The less certainty the teacher shows, the more certain he or she can be of realistically reflecting the difficulty of making decisions in these situations. Some will see an encouragement of relativism in this call for teachers to refrain from giving ready-made answers. An emphasis on ambiguity, however, is not only realistic, but imperative in deference to the moral self-determination of individuals (from Bayrhuber, 1989).

Case Study A. Microbiological Aid for Villages, by Peter J. Kelly (suggested for use after investigation H: Demonstrating the Effect of Streptomycin in the Small-Disc Test)

(The descriptions and questions are for students. Notes are provided after the questions suggesting possible answers and guides for discussion.)

You are a scientist working on a program to help remote, rural villages. You have been approached by representatives of the people of one of the villages who are concerned about the spread of diseases among their plants and livestock. They have little education but, interestingly, they are convinced that the diseases are caused by bacteria, and they want you to get rid of them.

Why Might the Villagers be Convinced that Bacteria are the Cause of the Diseases? (They may have heard that bacteria cause disease in a general sense and have applied the idea to their own circumstances. The term bacteria might have a meaning for them like "causes of disease" or "causes bad things," rather than a scientific meaning.)

What Would You Do First With the Representatives? (There is clearly a need to explain the causes of disease without offending or disheartening them.

You might start by finding out details of their problem from them and determining the extent of their understanding and misconceptions. Students could be asked to prepare a talk and deliver it to a group of students acting as the representatives and simulating their possible reactions.)

One of the representatives has, apparently, some understanding of biology, for she suggests you should undertake a practical investigation. She says the bacteria are carried in the air and you should collect air, grow the bacteria in it, and see if "antibiotic" kills them. You are surprised that she knows about "antibiotics," but you nevertheless ask her how she would do the investigation. She replies that you should expose jelly (nutrient agar?) to the air, allow bacteria to grow on it so that you can see them, and then place drops of antibiotic on it to see how effective it is in destroying bacteria.

Is There Any Value in the Investigation She Suggests? (The value is very limited; it would only show gross effects of the antibiotic and is not directly related to the villagers' problem. Scientifically, it lacks control and specificity. Students should attempt to explain the inadequacy of the suggestion in a simple, acceptable way.)

It turns out that the reason she knows about antibiotics is that the village was given stocks of penicillin (which was called "antibiotic") by an aid agency to help overcome bacterial infections secondary to an epidemic of influenza. The villagers perceive "antibiotic" as a kind of medicine. They still have some left.

How Would You Demonstrate the Value and Limitations of Using Penicillin to Overcome the Plant and Animal Diseases Affecting the Village? (Explain possible differences between plants, animals, and humans. If time permits, undertake specific, controlled investigations using actual diseased organisms.)

Are There Reasons Why Penicillin Should Not Be Used At All? (Penicillin is not a long-term answer to the problem and will, almost certainly, not be wholly effective; resistant strains will likely develop. The penicillin ought to be kept in case another flu epidemic occurs. This is likely, in the circumstances, to be a more conclusive way of ensuring that the discussion becomes more realistic.)

At this point one of the more fundamentally religious members of the village appears. He is angry. He says he represents the traditional beliefs of the village and argues that the diseases are part of nature. He contends that humans should not interfere with nature because it is acting against God's will.

What Are the Pros and Cons of the Argument Between Him and the Other Villagers? (Points raised might include: whether or not it is their God's purpose

to allow people to die; whether or not it is right for the village to survive; whether or not the diseases result from human sin. All such issues are ethical in character. They can provide a framework for action, but they do not solve the problem.)

Eventually this argument is resolved and the villagers wish to proceed, with your help, to eradicate the diseases.

How Would You Go About It? (Students should bear in mind that they should act as technologists as well as scientists. The villagers will be looking for relatively rapid progress and increased prosperity. Possible points for a strategy: destroy diseased crops and cultivate other ground; get diseases identified; use specific treatments for diseases; introduce resistant organisms; ensure there are no pollution effects if chemicals are used to treat plants or animals; set up breeding and other experiments with crops and livestock to establish long-term prosperity; educate villagers to ensure there are people with relevant expertise left in the village when you leave; attempt, by discussion, to reconcile traditional beliefs with scientific needs.)

Case Study B. An Enquiry into the Proposal to Map and Sequence the Human Genome, by Peter J. Kelly

One of the most significant decisions of our time is that to determine the position and sequence of each of the genes on the human chromosomes. This is an enormous task because each of us has about 100,000 genes and about 3×10^9 base pairs in the haploid genome. It also is an enormous responsibility because it will inevitably provide knowledge by which the basic form and function of *Homo sapiens* can be altered fundamentally--for good or ill.

Mapping and sequencing the human genome is an international endeavor with many countries taking part, some with large-scale projects, others with projects concerned with smaller, more specific aspects. There is a coordinating organization--the Human Genome Organization (HUGO)--to facilitate exchange of data and materials, encourage public debate, and provide appropriate information and advice on the scientific, ethical, social, and legal implications of human genome projects. It also is the case that there is competition between countries and, indeed, between individuals and organizations within countries.

Currently, the largest endeavor is in the United States, where, through programs established by the Department of Energy and the National Institutes of Health, more than three billion dollars will be spent. The basic objective is to speed up the rate of research, largely by focusing on the development of new technologies in the early stages of the project. At the previous rate of research, it would be about the year 2500 before a full map and sequence of the genome

was obtained. Now, biologists expect to have the complete map and sequence soon after the year 2000.

Many questions arise about the human genome project, some scientific, others to do with economics, ethics, and social issues. To consider and debate these issues, students need to look at them from different viewpoints. A means of facilitating this is to conduct a simulated enquiry or debate on the proposal "That the Human Genome Project be Established." This requires that two assessors and a chairperson be appointed--possibly staff members. Individual students or small groups should be designated as representative of various concerned people and should be required to prepare and debate their case before the assessors. The assessors should then summarize and evaluate the debate. The enquiry should be based on the ideas and procedures elaborated in Chapter 3.

The following selection of designated representatives could be used; others are possible, as well. Each designation is accompanied by statements on matters raised by such people in the debates that have accompanied the establishment of the actual human genome projects. The students should use the arguments for and against to compile their own views, aided, of course, by their own search for ideas and information.

Scientist

"An understanding of how humans function is one of the ultimate objectives of science, particularly of human genetics. The human genome project will help us meet this objective."

"We already know the basic structure of human DNA, so it is purely a matter of time before the map and sequence of human genes are produced--why not do it quickly?"

"Developments in automated technology and computers are now sufficiently advanced to allow the separation of the genome into its constituent parts. One field of science is inevitably driven by discoveries and inventions elsewhere."

"Science is value free. Scientists should be allowed to investigate anything--including the human genome. It is up to others to work out the implications. Science provides options, but individuals and society must choose among them."

"The project will bring prestige to scientists and their institutions, and

consequently more funding for science as a whole."
"The project will heighten competition among scientists and their institutions and will lead to dishonesty. This could have dangerous consequences."
"Money spent on the human genome project should be spent on research into more important topics such as the environment."

"The human genome project will lead to an emphasis on gene structure rather than developmental genetics, which is scientifically more important."

"You can never predict the eventual outcome of scientific research."

Physician

"The human genome project will help us overcome the 4,000 or more human ailments that have a single-gene component."

"The project will help us to eradicate diseases that have a multi-gene component--such as cancer and heart disease--and enable people to live longer and healthier lives."

"The knowledge gained from the project will enable genetic screening to be carried out more effectively."
"The human genome project raises the possibility that a person's genotype will become public knowledge; this could undermine the physician's relationship of confidentiality with patients."

"The information from the project will be too general to have real benefit to medicine. It would be better to concentrate research on specific genetic ailments."

"More money should be spent on treating patients and on overcoming social and environmental causes of illness than on academic research such as the human genome project."

Industrialist

"If we don't undertake the project, other countries will--and gain the profits."

"The project will lead to developments of great economic value in the pharmaceutical and other industries and will provide employment for many people."

"There is the possibility of developing new technology and techniques that

will reduce the cost of the project considerably."
"We need more time to think out the economic and other implications of the project."

"The money used in the project would be better spent on roads and other aspects of the economic infrastructure so as to support a wide range of industries."

"The results of the project should be made freely available to industry for economic exploitation."

"The government should own the patents to the knowledge about the genome (intellectual property) and regulate their use."

"Employers, insurance companies, or agencies of the state should have no rights to information about the genotypes of employees or clients."

Ethicist

"Human genome research will help us to understand ourselves and human nature more clearly, and enable us to behave more responsibly."

"Many human uses of genetic engineering resemble accepted forms of diagnosis and treatment that employ other techniques. The human genome project should provide thoughtful analysis, but should not be inhibitive."

"Morals change as understanding changes. Knowledge of the human genome will lead us to new moral criteria as acceptable as the ones we accept today."

"The problem is not to stop research on the human genome, but rather to educate the public."

"Science must take second place to our moral codes. We should first establish a moral code for dealing with the implications of human genome research before we embark on it."

"Any attempt to alter the natural state of human beings offends the integrity of human nature and should be resisted."

"Humans should not assume the prerogative of the Creator. No individual, group, or institution can claim authority to make decisions on behalf of the rest of the species; genetic engineering should be banned."

"Human genome research will support the ideas of biological determinism and lead to socially unacceptable behavior such as racism."

Case Study C. Conflicting Assessments of DNA Analysis in Prenatal Diagnosis (after H. Bayrhuber, 1989)

In this case, there is an irreconcilable conflict in the assessment of the objectives of a biotechnical process (recombinant DNA technology) in medicine. How is DNA analysis to be judged when it serves only as an aid for deciding for or against pregnancy termination, in other words, when an abortion would be considered if DNA analysis gave a clear indication of abnormality in the fetus?

In basic research, DNA analysis is used to map and sequence genes. It also serves to describe the functions of genes and to record the control mechanisms that govern them. Properties of the genotype can be directly analyzed by the examination of chromosomes or of DNA. They can also be ascertained indirectly through examination of the gene products.

In humans, DNA analysis can help to trace monogenic hereditary disorders, even those--such as Huntington disease--that occur late in life. DNA analysis also can help identify genetic predisposition to particular risks to which people are exposed at work or in the environment; these might include sensitivity to certain pollutants, medicines, or other substances. DNA analysis can also help to identify the presence of infections. Outside of medicine, it can be used to identify persons and to establish paternity.

An important area of application is prenatal diagnosis in genetic counseling. It involves an early determination of the presence or absence of hereditary disorders in the developing fetus. Watertight predictions are possible in some cases, and the number will continue to rise when the methods of DNA analysis can be applied. These methods allow, in effect, negative selection against disabled fetuses.

Advocates of DNA analysis as a basis for a decision for or against pregnancy termination maintain that it can spare a couple the tragedy of bearing a disabled child. The child can be spared a life of suffering and disability, because with the help of DNA analysis, fetal abnormality can be recognized early and the pregnancy terminated. The analysis also can offer a source of reassurance for parents worried without cause (naturalistic argumentation).

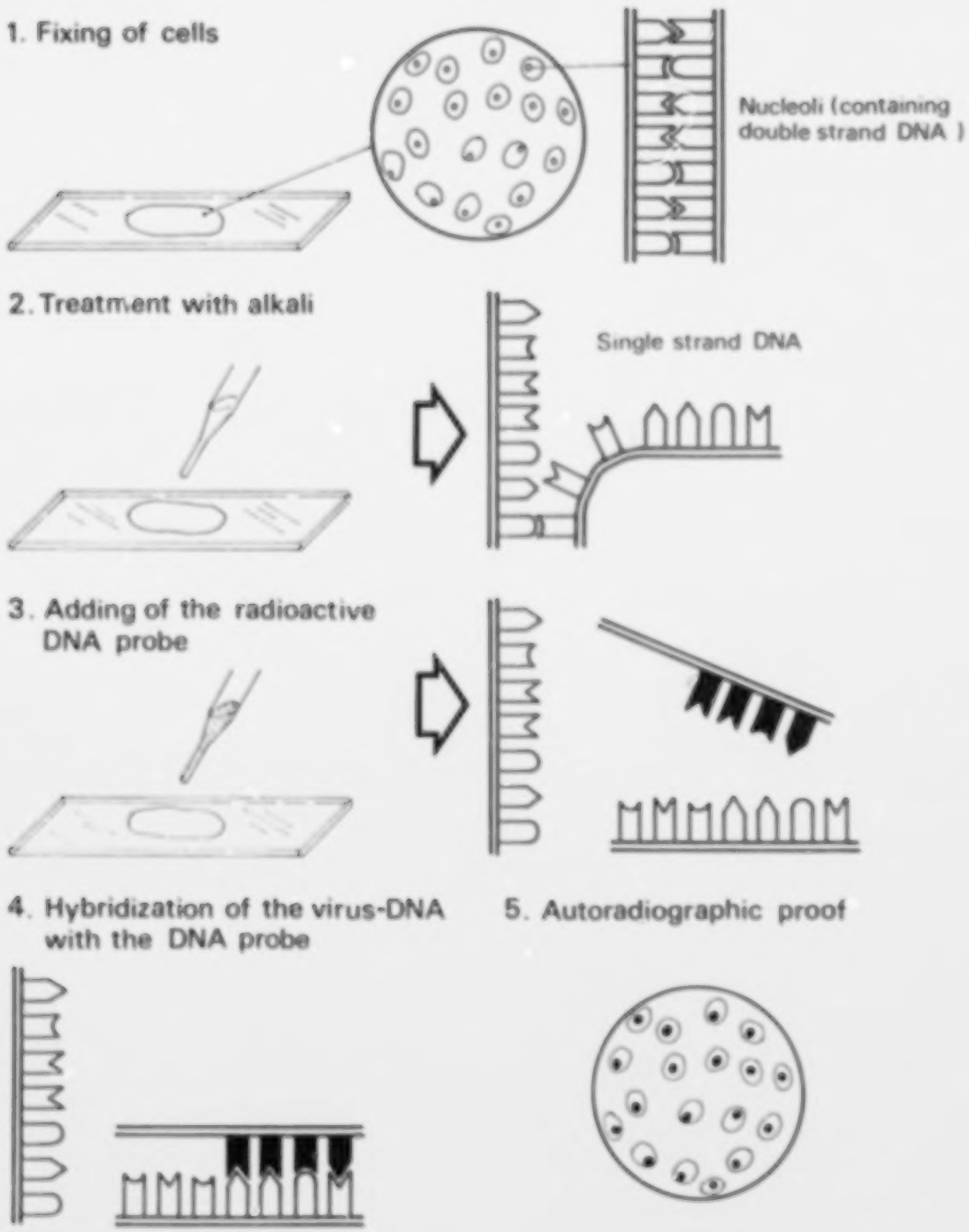
1. Fixing of cells
Nucleoli (containing double strand DNA)
2. Treatment with alkali
Single strand DNA
3. Adding of the radioactive DNA probe
4. Hybridization of the virus-DNA with the DNA probe
5. Autoradiographic proof

Figure 58. Demonstrating the presence of a virus by DNA analysis. This method is also used to detect hereditary disorders in prenatal diagnosis.

The contrasting view is that genetic selection is a form of euthanasia that disregards the value of human life. A further counter-argument is that the possibility of making clear predictions of disability could change the present, relatively tolerant attitude towards disability ("disability is suffering incurred through no fault of one's own; it requires help") in such a way that blame is assigned to the parents ("the disabled child need not have been born"). Pressure can be reckoned with; that is, pressure to terminate the pregnancy (personalistic argumentation).

These arguments by no means cover the problems of DNA analysis in their entirety. They simply illustrate the core of the conflict arising out of a juxtaposition of these two standpoints, which appear irreconcilable. For a detailed description of the naturalistic position and a discussion of personalistic arguments see, for example, Kuhse and Singer, 1985.

Helga Kuhse and Peter Singer argue that it cannot be completely wrong to take life--whether plant, animal or human--where there is no awareness of existence. A living entity without consciousness cannot, they maintain, suffer or be aware of deprival. This only applies, however, if there are no other reasons for not killing (in the case of plants, for example, the beauty of an old tree or the rarity of a type of orchid). When a being possesses consciousness, it should not be made to suffer without reason. If it can be expected to have pleasurable experiences in the future, it would be wrong to deprive it of these. This is reason enough for not killing a living being. The opposite would be the case if the being could be expected to suffer continually. The taking of life would then be justified. The authors state: "It is much worse to kill a being with self awareness and a sense of the future than to kill a being utterly incapable of entertaining any hopes or desires about what might or might not happen to it in the future" (p.131). They refer to Tooley, who considers the capacity to regard oneself as existent over time as a necessary condition for the right to live. According to Tooley, only a being that foresees an existence for itself can have the wish to continue living, and only a being that knows that it will continue to exist can have an interest in this continuation. Tooley's statement is founded on the naturalistic principle "that the wrongness of an action is related to the extent to which the action prevents some interests, desires, or preferences from being fulfilled" (p. 131). Kuhse and Singer conclude not only that pregnancy termination is morally justifiable, but also that termination of the child's life is justifiable in the first few months after birth.

The advocation of DNA analysis with abortion as the possible end is justified in relation to the well being of the parents and child, whereas condemnation of the procedure is justified in conjunction with human dignity,

that is, the absolute value of human life and the moral self-determination of the parents. The backgrounds of each of these justifications represent two different directions of ethical thought--the naturalistic and the personalistic; they are examined in more detail below.

1. Naturalistic justification

The ultimate end of moral behavior is the well being of human kind.

In terms of naturalistic justification, the following factors are among those that contribute to well being: good health, intact environment, good human relations, mental welfare, material goods.

This ethical position does not recognize, for example, the well being of the soul in a world to come as a particular manifestation of well being. The naturalistic conception accepts only those characteristics that one can grasp empirically. It does not differentiate in principle between human well being and that of other living things.

No distinction is made between the essence of humans and the essence of other creatures or things of nature. The naturalistic view of humans is characterized by statements that could similarly apply to animals, plants, and other natural phenomena. Examples would be: "Humans are a species of animal" or "Humans are a result of evolution." In naturalistic ethical terms, then, an action is right if it serves human well being, that is, satisfies interests, meets needs, and fulfills wishes.

Further characteristics of this form of justification are:

-Prescriptive propositions and value judgments are closely linked with experience. They should therefore be easier to impart and accept than criteria drawn purely from theory.
-Future experience is not handicapped by pre-definitions. Universal human moral growth is taken into account, as well as the moral growth of the students.
-Naturalistically founded criteria for action are revisable. Interests, needs, and wishes change in the course of social development.

2. Personalistic justification

Moral behavior serves ultimately to do justice to human dignity.

A reasonable person is distinguished by morals, that is, he or she is possessed of freedom and responsibility. Moral behavior is characterized

by anticipation of alternative action, recognition of the consequences of action, and the ability to make decisions. The essential properties of person are regarded as being of absolute value: they confer a dignity that is inviolable.

The essential properties of person also are unique in that they are not shared with any other organisms or natural phenomena. Thus, the personalistic view of human is characterized by the belief that there are differences in principle between the essence of humans and the essence of other living things or non-living phenomena in nature.

The personalistic ethical position justifies, among others, the following criteria for action:

-Each person shall enjoy unconditional protection by virtue of his or her absolute worth. The dignity of the person also renders human life inviolable.

-Humans may not be treated as objects (for example, by genetic optimization), nor may their normal self determination be hindered (for example, by subjection to considerations of usefulness or economic gain).

In practice, most people probably act in line with personalistic and naturalistic argumentation alternately. No problem arises if both types of justification lead to the same end. If, however, reference to well being leads to conclusions other than those drawn by the reference to human dignity, a schism ensues that cannot be bridged. The conflict cannot be resolved by the acquisition of more knowledge, nor by any pedagogical technique.

If a couple is expecting a disabled child, only the couple can make the choice between a naturalistically or personalistically justified course of action. Biology teachers cannot anticipate the decision for the students. Similarly, the debate over DNA analysis as an aid in deciding for or against pregnancy termination cannot be concluded by any argument, no matter how good it may be, because the points of reference taken to justify the arguments are irreconcilable.

Ethical Analysis

The strategy proposed by W.V. Mayer (after BSCS, 1988) is used here as a framework for ethical analysis with respect to DNA analysis in prenatal diagnosis (see

Chapter 3, "Social Implications of Biotechnology").

Step 1: Identify value-related concerns (interpretation).

The students should acknowledge that "DNA analysis" is a factual dimension in this connection. They also should recognize that the question of whether DNA analysis should be used in prenatal diagnosis to help decide about abortion is a value-related dimension.

Our knowledge about the genetic predisposition and the development of a child before and after birth is a factual dimension. The determination of the date when the fetus becomes a person is a value-related dimension.

Step 2: Identify the source within science of the value-related concerns (analysis).

An embryo or a fetus, originating from the combination of a human egg and sperm cell, develops into a child in a series of steps. Abortion means the termination of this development of a living being within a limited period of time after fertilization.

Step 3: Trace the implications (analysis).

One possibility is to claim that human life is sacrosanct from its very inception (combination of egg and sperm cell). Concerns for human dignity--the absolute and unique value of each human--would mean that abortion is strictly forbidden.

Step 4: Explain why the implications follow (argument).

Each human being has absolute value from its very inception. This would mean that the zygote already has the disposition to become a person and as such is characterized by absolute value (dignity). Genetic selection is a form of euthanasia that disregards the value of human life. Thus, abortion is regarded as murder, because it terminates human life during its development. The use of DNA analysis as a means of deciding for or against pregnancy termination is an acceptance of killing. For this reason it is regarded as a morally reprehensible action that should be avoided.

Step 5: Identify alternative values (analysis).

An alternative value could be the well being of the parents and of the child. The fulfillment of wishes and the satisfaction of interests is part of well being.

Step 6: Recapitulation of step 2.

The life of a couple who have borne a disabled child may be a physical and psychological torture, and the disabled infant will possibly lead a life of suffering.

Before the ninth month after birth, an infant is unable to consider "itself as itself, the same thinking thing, in different times and places" (John Lock, cited after Kuhse, H. and Singer, P., 1985, p. 132). Without consciousness of itself, it lacks an important precondition for having wishes or interests.

Step 7: Recapitulation of step 3.

The tragedy of bearing a disabled child, like the suffering of a disabled child, is contrary to well being.

Step 8: Recapitulation of step 4.

The abortion of a disabled fetus serves the future well being of its parents and prevents its own misery. The use of DNA analysis to decide for or against pregnancy termination promotes well being. For this reason, it is regarded as a morally good action. A being that does not even know that it exists, let alone having a conception of its own future, has no wish to live. It therefore has no claim to the preservation of its life. This also applies to new-born children until such time as they experience themselves as living in time.

Step 9: Critique.

Two critical statements are given below; they can be used to stimulate group discussions.

A. An argument against personalistic reasoning.

It is unjustifiable to give a human embryo or fetus the status of a person. There can be no question of this being taking responsibility and deciding in freedom. Life is a necessary condition for behavior as a person, and as the dignity of a person is inviolable, so too is life. But if embryos and fetuses are not persons, then they do not have a right to live.

B. An argument against naturalistic reasoning.

"From the moment the egg cell is fertilized, a life begins which is neither father nor mother but a new human being which develops independently. It would never become human if it were not so from this moment on. Recent genetic

research has given impressive confirmation of this fact, which has always been evident. It has shown that from the very first this creature has a definite structure, that of a human being, and more, of a specific human individual with all its characteristics accurately defined" (Sekretariat der Deutschen Bischofskonferenz, 1987).

Appendix 1

How to Produce Sterile Media or Solutions

Equipment

1 autoclave or pressure cooker
1 conical flask (300 ml to 2.0 l according to quantity)
culture tubes (or test tubes), sealed with cellulose bungs
petri dishes
pipette, 5 ml, sterile
pipetting aid
1 pH meter
1 spatula
1 set of scales
1 piece of weighing paper
1 pair of insulated gloves

Procedure

Agar Media:

1. Weigh out the individual components used for the manufacture of the culture medium onto a piece of weighing paper and place them into a conical flask. The quantities referred to below are for a 1 liter solution. If less culture medium is required, reduce the proportions of the individual components accordingly. The size of the conical flask depends on the amount to be produced; 300 ml conical flasks are, for example, required for 200 ml culture medium.

2. Swirl the preparation around until the soluble components have dissolved. Determine the pH and adjust it (if necessary) with 1 n NaOH or 1 n HCl, according to the values referred to below.

3. Seal the conical flask with a cellulose bung and autoclave in a pressure cooker at 121°C and 1 bar excess pressure for 20 minutes.

4. Remove the conical flask from the pressure cooker using protective gloves and cool the flask to approximately 50°C. This process can be accelerated by placing

the flask under running water. The temperature has been reached when the bare back of the hand can touch the outside of the vessel without an unpleasant sensation.

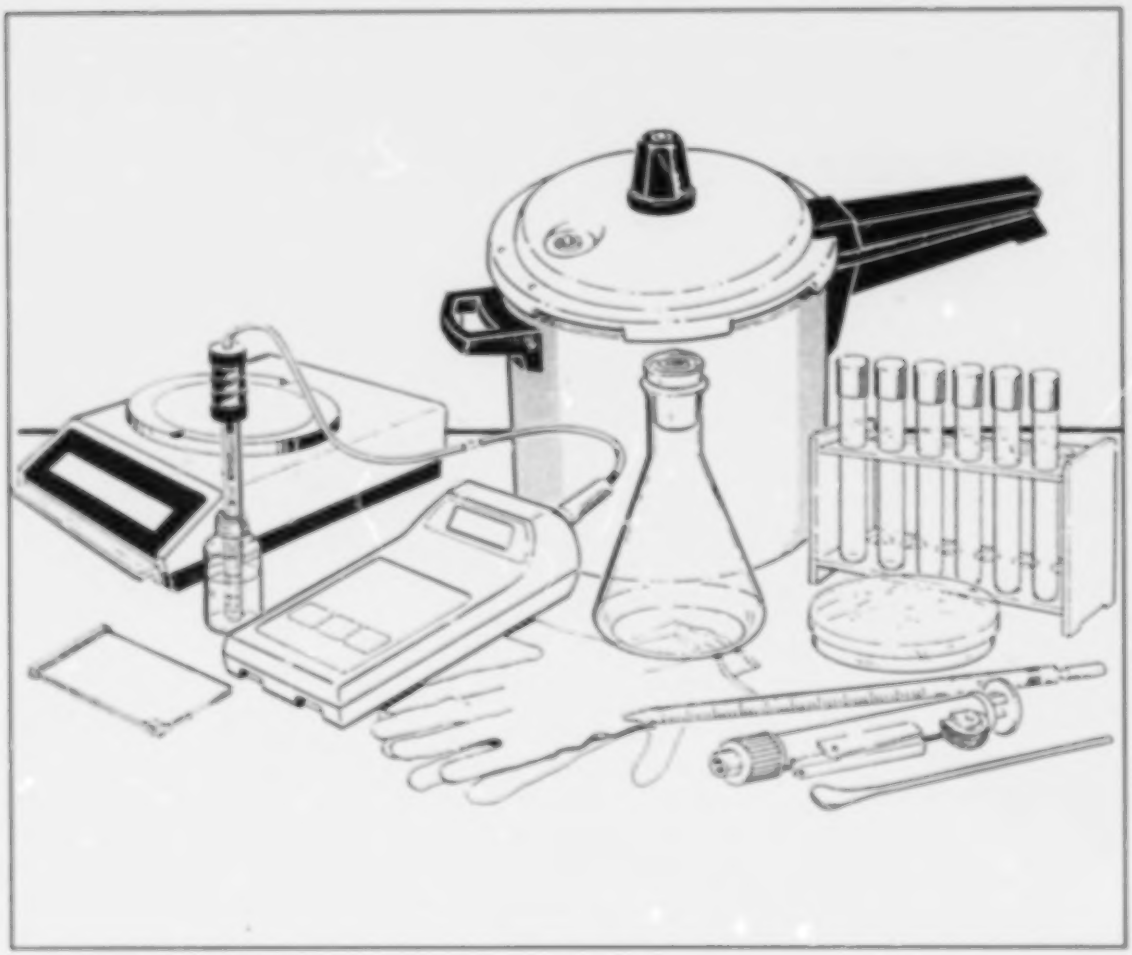

Figure 59. Required equipment.

5. Fill sterile plastic petri dishes with the still liquid medium so that the base of the petri dish is covered. Lift the lid of each petri dish only for a short period of time so that no germs from the air get into the culture medium.

6. Place the filled petri dishes in a safe place until the agar has set completely.

7. Inoculate the culture medium plates with material that contains bacteria, according to experimental requirements.

Liquid Broth Media:

1. Prepare the components required for the production of culture media according to the description for agar media, steps 1 through 4, autoclave, and cool down.

2. Fill culture tubes that have been sterilized in a drying cabinet (180°C, 3 h) with 5 ml of the culture medium, using sterile glass pipettes.

3. Inoculate the culture tubes with material containing bacteria, as described in the experiments.

Sterile Solutions:

1. Prepare the components required for the production of the solution according to the description for agar media, steps 1 through 4, autoclave, and cool down.

Media and Solutions

Basal Agar Medium (Investigations: A, E, F, G, I)

Components:		
glucose	10.0 g/l	
casein peptone	4.0 g/l	
meat extract	4.0 g/l	
yeast extract	0.5 g/l	
liver extract	0.5 g/l	
NaCl	2.5 g/l	
agar agar	15.0 g/l	(pH 7.2)

Basal Broth Medium (Liquid Medium--for overnight cultures) (Investigations: G)

Composition as for basal agar medium, however without agar agar.

Glucose Nutrient Agar (Investigation: E)

With ready-made medium:		
glucose	5.0 g/l	
nutrient agar	20.0 g/l	
or from individual component:		
glucose	5.0 g/l	
peptone from meat	5.0 g/l	
meat extract	3.0 g/l	
agar agar	12.0 g/l	(pH 7.0)

Malt Agar Medium (Investigations: B, H)

Ready made:		
malt extract agar	48.0 g/l	
or from individual components:		
malt extract	30.0 g/l	
peptone from meat	3.0 g/l	
agar agar	15.0 g/l	(pH 5.6)
autoclave carefully (10 min.)		

Minimal Agar Medium (Investigation: U)

Components:		
K_2HPO_4	3.5 g/l	
sodium citrate x 2 H_2O	0.5 g/l	
$MgSO_4$ x $7H_2O$	0.1 g/l	
$(NH_4)_2SO_4$	1.0 g/l	
glucose	2.0 g/l	
D,L-histidine	0.2 g/l	
D,L-arginine	0.2 g/l	
thiamine-HCl	0.05 g/l	
agar agar	15.0 g/l	(pH 7.2)

Nutrient Agar Medium (Investigation: E)

Ready made:		
nutrient agar	20.0 g/l	
or from individual components:		
peptone from meat	5.0 g/l	
meat extract	3.0 g/l	
agar agar	12.0 g/l	(pH 7.0)

Nutrient Broth Medium (Liquid Medium--for overnight cultures) (Investigation: U)

Ready made:		
nutrient broth	8.0 g/l	

or from individual components:
Composition as nutrient agar medium, however without agar agar.

Urea Agar Medium (Investigation: F)

Ready-made medium:		
A. urea agar (Christensen)	21.0 g/l	(pH 6.8)
B. urea	20.0 g/l	
distilled water	50 ml	

Sterilize part B with sterile filter; autoclave Part A and cool to 50°C; add part B to part A.

Components of Urea Agar (A):		
peptone from meat	1.0 g/l	
glucose	1.0 g/l	
NaCl	5.0 g/l	
K_2HPO_4	5.0 g/l	
phenol red	12 mg/l	
agar agar	12.0 g/l	(pH 6.8)

Vinegar Bacteria Medium (Liquid Medium--for overnight cultures) (Investigation: L)

Components of vinegar medium:		
peptone from meat	3.0 g/l	
yeast extract	5.0 g/l	
mannitol	25.0 g/l	

MS Agar Medium (Investigation: R)

With ready made medium:		
MS powder (basal salts with minimal organics)	4.7 g/l	
granulated sugar	30.0 g/l	
agar agar	8.0 g/l	
phytohormone solution (e.g., BAP, see below)	1 ml	(pH 5.8)

regulate with 1 m KOH.

BAP Medium (Investigation: R)

individual components:	
BAP (6-benzylaminopurine)	100 mg
1 n HCl	20 mg

Fill up to 100 ml with boiling distilled water (stock solution). Add 0.5 - 1.0 ml of this stock solution to culture medium (e.g., MS agar medium).

Buffer Reagent (Investigation: Q)

Individual components (1 m solution):

A.	K_2HPO_4	17.4 g/100 ml
B.	KH_2PO_4	12.9 g/100 ml

Composition (1 m buffer reagent):

Make a solution of bipotassium hydrogen phosphate (solution A) and potassium bihydrogen phosphate (solution B) separately, each time using 100 ml distilled water. Place 60 ml of solution A into a 100 ml conical flask and, using a pipette, adjust the pH to 7.5 with solution B.

Composition (0, 1 m buffer reagent):

Place 5 ml of solution A into a 100 ml conical flask with 45 ml distilled water. Place 5 ml of solution B into another 100 ml conical flask with 45 ml distilled water. Adjust the pH of dilute solution A to 7.5 by adding dilute solution B with a pipette.

20 Percent Domestos Solution (Investigation: R)

Composition:

'Domestos'-household cleaning fluid	20 ml

Fill up with distilled water to 100 ml. (Domestos is composed of saturated sodium hypochlorite solution).

Ringer Solution (Investigation: F)

Composition:

1 Ringer tablet

Fill with 500 ml distilled water and autoclave.

Salt Solution (Investigation: F)

Components:		
K_2HPO_4	3.8 g/l	
KH_2PO_4	1.2 g/l	
$MgSO_4 \times 7H_2O$	5.1 g/l	
NaCl	2.5 g/l	
$Fe_2(SO_4)_3 \times nH_2O$	0.05 g/l	
$Mn_2(SO_3) \times 4H_2O$	0.05 g/l	
Tween 80	1 ml/l	(pH 7.0)

Abbreviations

ATCC: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852,
DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1B, D-3300 Braunschweig, FR Germany
IPN: Institut fur die Padagogik der Naturwissenschaften - Institute for Science Education, Olshausenstrabe 62, D-2300 Kiel 1, FR Germany
NCSB: National Centre for School Biotechnology, Dept. of Microbiology, London Road, Reading, RG 1 5 AQ, GB

Chemicals

agar agar (MERCK No. 1614, SIGMA No. A 7002)
96 % alcohol (ethanol) (MERCK No. 818760, SERVA No. 11094)
70 % alcohol (ethanol) (MERCK No. 818760, SERVA No. 11094)
D,L-arginine (SIGMA No. A 4881)
BAP (6-benzylaminopurine) (SIGMA No. B 6894)
casein peptone (MERCK No. 2239, SIGMA No. C 9386)
'Domestos'-household cleaning fluid (containing sodium hypochlorite)
Eupergit C (from RÖHM GmbH, D-6700 Darmstadt, FR Germany)
$Fe_2(SO_4)_3 \times nH_2O$ (MERCK No. 3965, SERVA No. 20917)
glucose (MERCK No. 8342, SIGMA No. G 8270)
granulated sugar or sucrose (MERCK No. 7651E, SIGMA No. S 8501)
HCl (MERCK No. 9057, SERVA No. 34616)
D,L-histidine (SIGMA No. S H 7875)

Indian ink isopropanol (MERCK No. 9634, SIGMA No. 405 -7)
KH_2PO_4 (MERCK No. 4873, SIGMA No. P 0662)
K_2HPO_4 (MERCK No. 5099, SIGMA No. P 3786)
KOH (MERCK No. 9918, SIGMA No. P 1767)
lactase (SERVA No. 22075)
liver extract (MERCK No. 5347, SIGMA No. 201 -1)
malt extract (MERCK No. 5391E, SIGMA No. M 0383)
malt extract agar (MERCK No. 5398)
mannitol (MERCK No. 5982, SIGMA No. M 9647)
meat extract (MERCK No. 3979, SERVA No. 48020)
$MgSO_4 \times 7H_2O$ (MERCK No. 5886, SIGMA No. M 9397)
$Mn_2(SO_4)_3 \times 4H_2O$ (MERCK No. 5963, SERVA No. 28405)
MS-powder (after Murashige and Skoog) (SIGMA No. M 6899)
NaCl (MERCK No. 6404, SIGMA No. S 8888)
NaOH (MERCK No. 9137, SIGMA No. 930 -65)
$(NH_4)_2SO_4$ (MERCK No. 1211, SIGMA No. A 5132)
nutrient agar (MERCK No. 5450)
nutrient broth (MERCK No. 5443)
pectinase (SIGMA No. P 5146)
peptone from meat (MERCK No. 7214, SIGMA No. P 7750)
phenol red (MERCK No. 7241, SIGMA No. P 2167)
Ringer tablet (MERCK No. 15525)
sodium citrate (MERCK No. 6448, SIGMA No. C 7254)
sugar test strips
streptomycin (MERCK No. 10117, SIGMA No. S 6501)
thiamine-HCl (SIGMA No. T 4625)
tween 80 (polyoxyethylenesorbitan) (SIGMA No. P 1754)
urea agar (Christensen) (MERCK No. 8492)
urea (MERCK No. 818710, SIGMA No. U 1250)
washing-up liquid (containing tensides)
yeast extract (MERCK No. 3753, SIGMA No. Y 4000)

MERCK GmbH, P.O.B. 4119, D-6100 Darmstadt, FR Germany
Tel. (6151)72-0, FAX (6151)72-3368

SERVA Fine Bioch. Inc., Westbury, New York 11590, 200 Shames Drive, USA
Tel. (516)333-1575, FAX (516)333-1582

SIGMA Chemical Company Ltd., Fancy Road, Poole, Dorset BH17 7NH, England
Tel. (202)-733114, Telex 418242 - Sigma G

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Filmstrips and Videos

1 Biotechnologie (Biotechnology)

ZDF and Sudwestfunk have produced this new study series for adults who are interested in sciences. A survey of the current developments in this rapidly developing subject is provided in 13 programs.

What the individual programs contain:

The Institute for the Use of Films and Pictures in Science and Teaching (FWU) distributes this "BIOTECHNOLOGY" study program on 6 VHS video cassettes together with accompanying material. The cassettes can also be obtained separately: Institut fur Film und Bild in Wissenschaft und Unterricht, FWU, Bavaria-Film-Platz 3, D-8022 Grunwald.

Video 1 (Order No. 42 00637):

Mikroben - warum nicht? (Microbes - why not?)

Prof. Dr. Gerhard Gottschalk of the Institute for Microbiology at the University of Gottingen.

The fascinatingly colorful world of the smallest organisms: from the feared bacillus to genetic manipulation. Insight, overview, outlook.

Delikates - vom FlieBband (Delicacies from the Conveyor Belt)

Prof. Dr. Walter Hammes, Institute for Food Science at the University of Stuttgart-Hohenheim.

The culinary mass production of suitable bacterial strains by cultivation and composition: cheese and mold-ripened salami.

Video 2 (Order No. 42 00638):

Alkohol im FluB (Alcoholic Fermentation)

Prof. Dr. Armin de Meijere, Institute for Organic Chemistry and Biochemistry at the University of Hamburg.

Alcoholic fermentation by yeast...has always been practiced for wine and beer. Today: Alcholol from waste starch and Brazil's "alcohol farming."

Garer oder Atmer (Fermenters or Respirators)

Prof. Dr. Herhard Gottschalk, Institute for Microbiology at the University of Gottingen.

Different rates of metabolism in microorganisms.

Aus Mikroorganismen contra Mikroorganismen (From Microorganisms contra Microorganisms)

Prof. Dr. Gerhard Gottschalk, Institute for Microbiology at the Unviersity of Gottingen.

From Alexander Fleming's magic weapon penicillin to the made-to-measure broad-spectrum antibiotic.

Video 3 (Order No. 42 00639):

Syntheseanlage "Z" (Synthesis Plant "Z")

Prof. Dr. Maria Regina Kula, Institute for Enzyme Technology at the University of Dusseldorf.

Each observation of nature shows the quantity and diversity of substances produced (e.g. paints, smells) which remain beyond that reach of even the most brilliant science and engineering. It is fascinating how the natural "factory system," the metabolism of a protozoon, is used today in industrial production (example: citric acid, sodium glutamate).

Enzyme - ganz groB! (Enzymes - really great!)

Prof. Dr. Maria Regina Kula, Institute for Enzyme Technology at the University of Dusseldorf.

Enzymes are specialized biocatalysts. Far more than 2,000 of these catalysts are responsible for ensuring simultaneous linked reactions in cells. modern tanning and the production of washing powders make such enzymes work for them.

Video 4 (Order No. 42 00640):

Raum zum Leben, Raum zum Schaffen: Bioreaktoren (Space to Live, Space to Create: Bioreactors)

Prof. Dr. Christian Wandrey, Atomic Research Plant Julich Ltd., Institue for Biotechnology, Institute 2.

Enzymes require a suitable environment for their work. The film shows the role of biological procedures using the example of amino acids: developing industrial production conditions by imitating nature (fermenter technology).

Klar wie Abwasser (As clear as Sewage)

Prof. Dr. Christian Wandrey, Atomic Research Plan Julich Ltd., Institute for Biotechnology, Institute 2.

Effluent technology is based on microorganisms. Aerobes are the hardest workers in our sewage works at present. Their high oxygen requirements cause enormous energy costs. Might techniques using anaerobes be a way out? They do not require expensive oxygen and produce biogas.

Video 5 (Order No. 42 00641):

Gene zum Anfassen (Genes you can touch)

Dr. Hans-Joachim Fritz, Max-Planck-Institute for Biochemistry, Martinsried/Munich.

One of the best kept secrets of nature has been discovered: the genetic code. Molecular geneticists are no longer satisfied with decoding, they interfere with gene combinations...and become authors themselves, so to speak.

Planspiele mit Genen (Planning Games with Genes)

Prof. Dr. Konrad Beyreuther, Institute for Genetics at the University of Cologne.

The film describes an incredible puzzle: Geneticists manipulate genetic commands so that cells produce substances which are foreign to thme (e.g. human insulin from coli bacteria).

Video 6 (Order No. 42 00642):

Manipulierte Pflanzen - und dann? (Manipulated Plants - what next?)

Dr. Bruno Gronenborn, Max-Planck-Institute for Research into Breeding, Cologne-Vogelsang.

Plant genetics has a fascinating future. Being able to intervene in the tempo of the development of the genetic program or being able to add a new element - for example resistence to certain weed killers - is an advance, however it is not without its problems.

The film provides insights into current research into breeding.

Selbst oder Nicht-selbst (Self or non-self)

Dr. Hans-Joachim Fritz, Max-Planck-Institute for Biochemistry, Martinsried/ Munich.

Immune biology was a milestone in the history of medicine which is still significant today. A new perspective: endless sources of antibiodies due to the fusing of different types of cells.

Quo Vadis?

Prof. Dr. Gerhard Gottschalk, Institute for Microbiology at the University of Gottingen.

A mood of restlessness in biotechnology, more specifically in genetic engineering. Economic expectations are irrevocably lined with ethical responsibility. The aims and possibilities of this blossoming area of science and its links with ethical implications are shown.

2 Gentechnik - Grundlagen und Anwendungen (Genetic Engineering - Fundamentals and Perspectives)

by Gabriela Bongers, and Prof. Dr. Jurgen Bongers

Hagemann transparencies, Dusseldorf 1989
Hadu-Hagemann, Lehrmittel- und Verlagsgesellschaft mbH, KarlstraBe 20, D-4000 Dusseldorf 1. Order No. 17 22 70

The conception of the transparency file makes a discriminating methodical approach for lower secondary schools (11-16 year-olds) and older students (16-18 year-olds) possible. This is why specific worksheets and information sheets have been produced for both groups of students. A glossary defines the most important specialist terms. Specialist information outlines current developments.

3 Bakterien und andere Mikroorganismen (Bacteria and other Microorganisms)

by Heinz Fischer, Hagemann transparencies, Dusseldorf 1978
Hadu-Hagemann, Lehrmittel- und Verlagsgesellschaft mbH, KarlstraBe 20, D-4000 Dusseldorf 1. Order No. 17 21 72

Twelve transparencies, some of which consist of several layers, which describe the most important things about microorganisms by the use of examples, and in a manner which is easily understood by secondary school pupils of all ages (11-18 year-olds) have been collected in this file.

4 Mikroorganismen als Krankheitserreger (Microorganisms as Pathogens)

by Heinz Fischer, Hagemann transparencies, Dusseldorf 1978
Hadu-Hagemann, Lehrmittel- und Verlagsgesellschaft mbH, KarlstraBe 20, D-4000 Dusseldorf 1. Order No. 17 21 73

Secondary school students (11-18 year-olds) are informed about the basics of human infectious diseases in the followng file by ten transparencies some of which are composed of several layers and provide examples, and questions for problem solving in lessons. They are thereby enabled to understand and assess such illnesses, to understand what the doctor says, to be able to consciously follow the doctor's prescription and to prevent infection by sensible behavior.

5 Biotechnologie/Gentechnik (Biotechnology/Genetic Engineering)

Transparencies, Copy Models and Text Book. Frankfurt, 2nd Impression 1989

The materials are provided free of charge by the Chemical Industry Fund, Fond der Chemischen Industrie, KarlstraBe 21, Postfach 11 19 43, D-6000 Frankfurt 11.

Order: Transparency Series 20.

Transparencies on the following themes have been collected: areas of biotechnology, history of biotechnology, biotechnology and genetic engineering (the genetic apparatus fo the cell, new combinations of DNA, microbiological techniques, fermentation and immobilization), areas where biotechnology and genetic engineering can be used, safety considerations.

6 Infection of Clover by Root-nodule Bacteria

P.S. Nutman, C.C. Doncaster, P.J. Dart, London. 1973. Black/white, sound (commentary English), 165 m, 15 min.

Institut fur den Wissenschaftlichen Film (IWF), Nonnenstieg 72, D-3400 Gottingen. Order No. W 1236.

The infection of clover by nodule bacteria. The formation of root-nodules as centers of molecular oxygen fixation in a physiologically usable form. The development of infection. With time-lapse photography.

7 Zellteilung - Escherichia coli (Cell Division - Escherichia coli)

T. Hard (IWF), Gottingen. Prod.: 1981, Publ.: 1982. Color, silent, 16 m, 1 1/2 mins.

Institut fur den Wissenschaftlichen Film (IWF), Nonnenstieg 72, D-3400 Gottingen. Order No. K 129.

The extension of bacterial cells, abstriction, simple splitting, formation of a bacterial lawn on agar-agar. Time-lapse photography.

8 Pflanzen nach MaB - Landwirtschaft 2000: Nahrung und Energie im UberfluB? (Plants made-to-measure - Farming in the year 2000: A Superabundance of Food and Energy?)

H.E. Weitzel, Daxweiler (Hunsruck). 1978. Color, sound (commentary German), 328 m 20 min.

Institut fur den Wissenschaftlichen Film (IWF), Nonnenstieg 72, D-3400 Gottingen. Order No. W 1708.

Selection according to resistance produced by crossing resistant wild varieties is demonstrated with reference to tomatoes and cucumbers. "Archives of plant genes," so-called gene banks, guarantee a rich variety of seed for crossing experiments. The fusing of protoplasts followed by the fusing of nuclei promises to become a new method of plant breeding. Professor D. HeB, Hohenheim, explains his aims concerning transferring genes for the use of atmospheric nitrogen to cereals.

9 Herstellung einer Reinkultur am Beispiel von Escherichia coli (The Producton of a Pure Culture Demonstrated by Escherichia Coli)

N. Pfenning, E. Siefert, Gottingen. IWF, Gottingen. Prod.: 1975 and 1979, Publ.: 1980. Color, sound (commentary German), 62 m. 6 min.

Institut fur den Wissenschaftlichen Film (IWF), Nonnenstieg 72, D-3400 Gottingen. Order No. C 1345.

The representation of a microbiological method. Starting with unpurified effluent from a sewage works a pure culture of *E. coli* bacteria is produced using optical controls and a selective indicator culture medium (EMB agar) after two dilution streaks have been carried out. A culture of the strain is incubated on slant agar.

10 Ermittlung einer Wachstumskurve am Beispiel von escherichia coli (Determination of a Growth Curve Demonstrated by Escherichia coli)

N. Pfenning, E. Siefert, Gottingen. IWF Gottingen. Prod.: 1975 and 1979, Publ.: 1980. color, sound (commentary German), 96 m, 9 min.

Institut fur den Wissenschaftlichen Film (IWF), Nonnenstieg 72, D-3400 Gottingen. Order No. W 1346.

The representation of a microbiological method. The increase of the bacterial cell mass is determined in a static culture over 7 minutes by measuring clouding. The doubling of the cell mass during the exponential growth phase is read from the growth curve which is represented in a simple logarithmic way.

11 Bestimmung der Zellzahl in einer Kultur am Beispiel von Escherichia coli (Determination of the Number of Cells in a Culture as deomonstrated by Eschrichia Coli)

N. Pfenning, E. Siefert, Gottingen, (IWF), Gottingen Prod.: 1975 and 1979, Publ.: 1980. Color, sound (commentary German), 102 m, 9 1/2 min.

Institut fur den Wissenschaftlichen Film (IWF), Nonnenstieg 72, D-3400 Gottingen. Order No. W 1347.

Representation of microbiological methods:

1. the microscopic determination of the entire number of a mature culture with a counting cell

2. the determination of the number of live cells by counting bacterial colonies on agar plates. The computational determination of numerical values.

12 Pflanzen nach MaB (Plants made-to-measure)

H.E. Weitzel, FWU, Grunwald, 1985.

Institute fur Film und Bild in Wissenschaft und Unterricht, FWU, Bavaria-Film-Platz 3, D-8022 Grunwald.

The film is concerned with experiments carried out by groups of scientists to breed new plants by crossing, so as to be able to feed mankind better. Plants are also bred to resist pests and illnesses. Gene banks provide the seed. New breeding methods are being developed like the fusion of plant cells to unite the genetic material of quite different plants and produce completely new species. Various research methods and procedure are described very clearly and well. Research aims, results, successes, and failures too are presented.

Chapter 5

CONSIDERATIONS FOR IMPLEMENTATION

Barriers to Teaching Biotechnology in Schools

Although there are several compelling arguments for the inclusion of biotechnology in the school curriculum, there also are a number of limiting factors that require consideration. These include obvious constraints such as the lack of teacher awareness and of appropriate resources, and not-so-obvious, though equally significant constraints such as the structure of the curriculum as a whole and outdated assessment procedures that militate against innovation.

1. *Teachers' Lack of Awareness of Biotechnology.*

Until recently in the U.K., the majority of biology teachers have been graduates of pure aspects of the subject such as botany and zoology, and consequently have little or no experience with the basic microbiological and biochemical techniques that are fundamental to biotechnology. In addition, their subsequent teacher-training courses have contained little or no reference to biotechnology. In the United States, the average age of biology teachers--approximately 40--indicates that many teachers were trained before the ascendance of molecular biology and immunology.

The first centrally funded U.K. initiative concerning biotechnology in schools was undertaken in 1983-84. The overall brief was an assessment of the level of awareness of biotechnology in schools. One of the conclusions of the ensuing report (Beetlestone and Teasdale, 1984) was that "While many biology teachers know of the potential economic importance of biotechnology and are interested in incorporating aspects of it into their teaching, few have the training, experience, or educational incentive to give pupils an awareness of the biological technology system."

Given this general inability on the part of the teachers to explain the implications of biotechnology to pupils, the following observations from the same report on the awareness of pupils are not surprising:

> There was a low ability to discriminate between correct statements about biotechnological industrial realities and statements describing possibilities far beyond the current "state of the art" under laboratory conditions. There was also some evidence that the inclusion of such words and phrases as artificial, cloning, gene therapy, genetic engineering, and industrial products in any of these statements tended to engender disapproval of the possibility described by the statement.

The problem of inadequately prepared teachers is universal. As Bayrhuber (1989) remarks:

> Teachers throughout the world are not adequately prepared for the task of teaching biotechnology. Frequently they lack the microbiological training which would facilitate the use of microorganisms. In-service training programs are urgently required to enable the teachers to deal confidently with microbes under suitable conditions (including climatic conditions) and to remove their unfounded antipathy towards using those organisms.

2. *Shortage of Resource Materials.*

Resources for teaching aspects of biotechnology were nonexistent until very recently, although exercises in practical microbiology have been available for some time. In the U.K., some resources for teaching biotechnology have arisen from a variety of sources over the last few years. Appendix 1 summarizes the contents of a number of the written resources. A number of experimental kits are available from school suppliers in the U.K. These focus on areas such as enzymology, microbial culture, tissue culture, and food biotechnology. A variety of fermenter systems also is available, although for most schools these are prohibitively expensive. In America, the Cold Spring Harbor Laboratory has devised a laboratory course in recombinant DNA aimed at school teachers and pupils. The course culminates in the production and analysis of recombinant DNA molecules. A common feature of practical activities in the areas of biotechnology and microbiology is that they are demanding in terms of preparation, often requiring considerable amounts of apparatus and reagents. Even in developed countries, where the provision of technical support in schools is relatively generous, this consideration often leads to limited experimental work by the pupils. Little information is available on teaching resources in the developing world, although one assumes that these are negligible because of the number of requests for help received in the U.K. and other developed countries.

3. *Teaching Methods.*

The problem of teachers' lack of subject expertise is compounded by their limited grasp of innovative teaching methods. As Vohra (1989) explains,

> ...biotechnology is a multidisciplinary and a complicated field, which most school teachers trained as straight subject teachers find it rather difficult to handle in the classroom situation. Biotechnology combines science and technology and therefore interacts freely with the socioeconomic, political, and ethical issues of the community in which it is practiced.

In addition, new material often is taught for its own sake, with little consideration of how it reinforces the major, enduring themes of biology, or of science as a process of rational inquiry.

Although teachers are competent at traditional instructional methods in teaching science as a body of knowledge, they often lack experience and confidence in developing multidisciplinary approaches involving several different departments. The same is true of their grasp of other innovative approaches such as problem solving or teaching through discussion of controversial issues, which are important for effective education about biotechnology.

4. *Lack of Interaction with Industry.*

Instruction about biotechnology that does not include input from industry is not likely to be worthwhile. School/industry links are notoriously difficult to establish on a wide scale, but this problem must be addressed if children are to gain realistic insights into the nature of biotechnological processes.

There are several levels of involvement of school/industry links, ranging from visits to industries for pupils to secondments of teachers to work in industry for a defined period. The middle ground includes productive working parties consisting of teachers and industrialists who meet on a regular basis to produce appropriate teaching materials or strategies.

The factors that severely limit the number of school/industry links include the reluctance of industry to spare the necessary person hours for something that is not recognized as of primary importance, and a somewhat negative attitude towards industry on the behalf of teachers.

A more specific drawback that has recently become evident in the U.K., where there is much interest in school/industry links, has been the plethora of agencies that exist to promote them. The following problems are noteworthy:

A. Newcomers, especially representatives from industry, are confused and use this as an excuse for a low level of involvement.

B. Companies receive uncoordinated requests for help from a number of agencies and individual educational establishments.

C. Inexperienced schools and companies can be misled by an overzealous agency into thinking that their particular activity is the only workable model for school/industry collaboration.

5. *Lack of Formal Assessment.*

The adoption of innovative curriculum material by teachers is, to a large extent, dependent on the whim of examining bodies. Examination syllabuses must include the relevant topics and concepts if biotechnology is to be taught in schools. Furthermore, if teachers are to include biotechnology in the curriculum, then examination papers must include questions on it. Although there are encouraging signs in this regard in several countries, it is doubtful that these developments are universal.

6. *The Traditional Nature of Science Teaching.*

A further barrier to the incorporation of biotechnology into the curriculum is the discipline-bound, academic nature of traditional science education. As Henderson and Lally (1988) explain:

> One doesn't have to teach science in school for very long to realize that much of it is, and has been, concerned with facts and principles; consequently it is very knowledge orientated. As recently as 1980, it was publicly argued that pupils up to the age of 16 should receive a science education based on "facts." Some of the reasons for this are obvious. We are all constrained by the examination syllabuses in the upper school, and by our own scientific training. It would have fitted us well for a scientific career. It would also have given us the key conceptual frameworks for our own discipline, and taught us a few research techniques. However, it probably didn't encourage us to think about science as well as training us in science. It does not necessarily follow that such training will serve us equally well as science educators.

These views are reinforced in the U.K. by a recent report from the Department of Education and Science (1985), which indicated that too much time was spent on accumulating facts and principles, much of them being irrelevant. The report found most experiments to be "laboratory based and divorced from the present-day world," while suggesting that "the balance of practical work should be more towards solving problems." Reports from the United States (AAAS, 1989; National Research Council, 1989), show that similar problems exist there.

7. *Overcrowded Curricula.*

The inclusion of new material in school curricula that already are overcrowded is a perennial problem. During the past twenty years, the amount that pupils are expected to remember for examinations has increased substantially. There are now severe pressures on time within the existing framework of the school curriculum and hence little opportunity to expand upon the social and economic importance and application of the science subjects

studied. This clearly has implications for the inclusion of biotechnology in the curriculum. The problem of outdated and overcrowded syllabuses is by no means endemic to the U.K. Brumby (1987) comments that "all around the world there is a growing realization that a major re-evaluation of the goals and content of science education is required to prepare students to live in the 1980s and 1990s." In the United States, McInerney (in press), has written about the "horizontal expansion" of curricular topics, which precludes an articulated "vertical integration" of major concepts.

8. *Public and Parental Acceptance.*

Because biotechnology raises contentious social and ethical issues, its inclusion in the school curriculum is likely to meet with opposition from certain sectors of the public and from some parents. It is probable that many of these misgivings arise from ignorance or misinformation, (OTA, 1987; Research and Forecasts, 1987), but they are nonetheless significant.

This is illustrated by the recent success of pressure groups in, for example, the United States of America and West Germany in restraining biotechnology in research and development. If the public perception of biotechnology can lead to such lengthy delays in official approval for industrial projects, it is at least possible that the introduction of biotechnology in schools will meet similar resistance.

Overcoming the Barriers

1. *In-service and Pre-service Training.*

During the last five years, several projects have been initiated to meet the needs of teachers for in-service training in biotechnology. In the U.K., the National Centre for School Biotechnology (NCSB) has recently coordinated a centrally funded initiative involving three centres (the Universities of Reading and Sheffield, and Southbank Polytechnic, London), the object of which is to develop materials for in-service training of teachers. The "TRIST Biotechnology 13-18 Initiative" provided a considerable amount of useful training material, and teacher-training facilities based on these materials are currently provided at the NCSB and at the University of Sheffield, Division of Education. NCSB courses are run on both a local and a national level and encompass a broad range of biotechnological activities, from aseptic technique to some aspects of recombinant DNA technology. The approach at the University of Sheffield has been to use biotechnology as a vehicle for introducing teaching strategies based on problem solving and controversial issues.

Other countries in the developed world also are fostering the training of teachers in biotechnology. In the United States of America, for example, the Cold Spring Harbor

Laboratory has developed courses in recombinant DNA technology for school teachers, and more recently has opened the "DNA Learning Center," which provides courses and study facilities for pupils as well as teachers. The Human Genetics and Bioethics Laboratory at Ball State University, Muncie, Indiana, trains approximately 25 teachers each summer in analysis of ethical issues and in teaching strategies for secondary classrooms. Courses for teachers in recombinant DNA techniques also have been provided in Denmark, with the help of a limited amount of central funding, while more general overviews of biotechnology have been evident at biotechnology conferences for teachers in Kiel, West Germany, and Nijmegen, Netherlands.

Although these initiatives are encouraging, there is still a great deal to do. Some consideration has been given to the implications of biotechnology for science teaching in the developing world (Padolina, 1987), but no information is available on training initiatives for teachers. In general, greater effort is required to improve teachers' knowledge of biotechnology and to provide them with experience in practical techniques.

2. *Resources.*

Appendix 1, referred to in the previous section, indicates that some resources are available for teaching biotechnology, in the U.K., and there are a small number of specialized courses, as well, in a variety of developed countries. In the United States, the Biological Sciences Curriculum Study (BSCS) has produced materials on biotechnology for pupils and teachers. These include a four-week module titled *Advances in Genetic Technology*, and laboratory investigations that are integral to the full-year BSCS programs *Biological Science: An Ecological Approach* (BSCS Green Version) and *Biological Science: A Molecular Approach* (BSCS Blue Version).

As is the case for teacher-training facilities, initial efforts in the production of resource materials are encouraging, but insufficient, and teaching resources in the developing world are apparently nonexistent. There is clearly a need for the development of protocols that describe affordable classroom simulations of endemic biotechnology practices (or potential practices). Furthermore, there is still much room for the production of skillfully written, comprehensive materials that convey a basic understanding of the mechanisms and implications of complex biotechnological processes.

3. *Involvement of Industry.*

Although the majority of industrialists offer little more than lip-service to effective links with schools, most are in favor of such links in principle. Teachers' attitudes toward industry, on the other hand, are traditionally skeptical. The reluctance of industry to join in collaborative ventures in curriculum development helps to perpetuate these feelings. The solutions to these separate problems are, therefore, related. Converting favorable noises from industry into active participation does, no doubt, help to create a more positive

image of industry among teachers.

Marsden (1988) summarizes the rationale for the establishment of school/industry links as follows:

> As the benefits of links are more obvious for the education side, the onus for developing activities further almost invariably rests with schools and colleges. To do this, it is very important for schools to have clear objectives and well-marshalled arguments for why business should get involved. There are equal benefits for business as for education, but they do need spelling out and then selling....All businesses, even small ones, benefit from good public relations. Staff recruitment, day-to-day operating, and marketing are all enhanced by a favorable local reputation. Local schools and colleges are ideal places through which to get better known, to be regarded as valuable part of the community, a provider of useful products or services, and as a desirable place to work.

These observations are particularly relevant to biotechnology industries at the moment. In the U.K. and other EEC countries, the predicted, large number of school leavers in the next decade has severe implications for industrial recruitment, especially in high-technology industries. Personnel charged with promoting links must, therefore, make sure that companies are fully aware of this situation, and that their collaboration with schools can help to alleviate it.

School/industry links that do exist usually involve large multinational companies with separate education budgets, rather than smaller, independent concerns that are often characteristic of biotechnology. If the demographic changes predicted over the next few years are not enough to persuade a far wider range of companies to build closer links with schools, governments will be under increasing pressure to intervene with, for example, tax incentives to provide industrial secondees to schools. Such intervention also could alleviate the aforementioned problems that arise when several different agencies promote links between schools and industry.

In addition to the establishment of a "favorable local reputation" referred to above, it is particularly important that biotechnology industries, which often work in sensitive areas, promote a positive public attitude about their activities. Educators and policymakers must therefore make it clear to industrial representatives that there are few more effective ways to achieve this than through collaboration with schools.

4. *Biotechnology and Curriculum Innovation.*

There is consensus among biologists and science educators that biotechnology can be applied across a wide range of school subjects. These opportunities have been

advocated since as long ago as 1981, when the Royal Society Working Party on Biotechnology (1981) made the following recommendations:

> We would wish to see important aspects of biotechnology, both theoretical and experimental, introduced into biology, chemistry, and physics syllabuses. We believe that this can be done without removing any essential element of current syllabuses or further overloading them, through judicious use of material, for example, in microbiology, which exemplifies principles, develops skills, and generates awareness of applications in industry.

These views were later reiterated and developed by the Association for Science Education (ASE) (1986), which stated:

> Biotechnology should be a distinct part within the science curriculum, since it is the outcome of multidisciplinary research and development. If possible, it would be most appropriate to have a biotechnology unit within a science syllabus. During this unit, the various themes that have occurred during the other lessons (science, technology, home economics, etc.) could be examined in more depth and provided with a cohesiveness within a biotechnological context.

Recent developments in the U.K. promote development of balanced science courses to grade 16+ in place of the separate disciplines of biology, chemistry, and physics. This movement clearly favors the strategy for biotechnology teaching suggested by the ASE, and there already are examples in the U.K. of biotechnology courses that link science with courses in design and technology and in home economics (Buckland, 1987; Yorke, 1987).

Biotechnology education can help overcome the difficulties of attracting girls to technological subjects. A 1985 survey by Her Majesty's Inspectorate (1985) found that "technology courses were heavily biased in favor of physics and engineering. Valuable as this work is, more attention should be given to the development of technological work which reflects the importance of biology and chemistry and which is of intrinsic interest to both boys and girls." Such statements provide a strong case for the inclusion of biotechnology in the curriculum, as it is likely to prove favorable to the expectations and attitudes of girls to technology in general. Furthermore, unlike many other areas of technological activity, several aspects of biotechnology have firm roots in the developing world and are directly relevant to the school populations of those countries.

5. *Examinations and Examination Boards.*

In many countries, the adoption of innovative curriculum material ultimately is dependant on the whim of the examining bodies. If biotechnology is to be taught in

schools, the topic must be included in relevant examination syllabuses, and its inclusion in the curriculum must be justified by pertinent questions in examination papers. One must review the teaching opportunities offered by various syllabuses to assess the degree of commitment of U.K. examining bodies to increasing biotechnological awareness. Although the increased opportunities offered by these and the new U.K. "A" level syllabuses for the teaching of biotechnology are encouraging, the extent to which the new examination papers encourage teachers to include biotechnology in the curriculum remains to be seen.

Persuading examination boards to set questions on any particular area of the curriculum is no easy task, and the problems are exacerbated when the subject in question is new and unfamiliar. Furthermore, examination boards are commercial concerns, and market forces therefore affect decisions about the content of examinations.

An effective lobbying tactic was adopted recently by the U.K. government's Industry Education Unit. A working party under the auspices of this unit was established to examine ways to promote industrial awareness in biological studies in school. (Similar groups dealt with the other sciences and mathematics.) The group set up workshops to generate examination questions on biotechnology. The chief examiners were brought together with industrialists and given the task of writing questions about biotechnology. These meetings proved very successful and emphasized the examiners' need for access to information and data on industrial processes.

Safety Issues

Developments in biotechnology have been accompanied by considerable debate about associated ethical and safety issues. Safety concerns arise primarily because most biotechnological processes use microorganisms, some of which can affect humans. Smith (1987) lists the main areas of consideration for safety aspects specific to biotechnology as follows:

A. Pathogenicity: potential ability of living organisms and viruses (natural and genetically engineered) to infect humans, animals, and plants and to cause disease.

B. Toxicity and allergy associated with microbial production.

C. Other medically relevant effects: increasing the environmental pool of antibiotic-resistant microorganisms.

D. Problems associated with the disposal of spent microbial biomass and the purification of effluents from biotechnological processes.

E. Safety aspects associated with contamination, infection, or mutation of processed strains.

F. Safety aspects of the industrial use of microorganisms containing *in vitro* recombinant DNA.

Because many classroom activities designed to simulate biotechnological processes involve the use of microorganisms, these considerations are relevant to schools. Teachers supervising such activities must have appropriate experience with sterile techniques and a general understanding of the potential hazards of working with microorganisms.

In the U.K., there have been several opponents to the use of microbes in school; the opponents include nonspecialists, science advisers form the education authority, and professional pathologists. As a consequence, the teaching of microbiology in schools has a checkered history. In the early 1970s there was a flurry of interest with the introduction of Nuffield science courses. This interest soon dissipated, however, largely as a result of one or two isolated, but over-publicized, cases of bad microbiological practice in schools. During the past few years, interest in school microbiology has reemerged largely because of the focus of interest on biotechnology.

Local Education Authorities in the U.K. still differ, however, with regard to acceptable codes of microbiological practice in schools. The authorities have given little attention to codes of practice in the wider context of biotechnology; extant advice is

concerned solely with microbiological considerations. Given the U.K. example, the introduction of biotechnology in schools would be facilitated by the introduction of codes of practice in microbiology and other aspects of biotechnology--recombinant DNA techniques, for example--that are both accessible and universally applicable. Although it is likely that the general principles of such recommendations would be universally applicable, they might require minor revision to accommodate local differences in materials and methods.

Until such codes of practice are available, however, the reader is referred to several sets of existing recommendations from different countries, some extracts of which are included in appendix 2. It is critical that teachers heed such recommendations when using the classroom activities provided in chapter 4.

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Appendix 1

Selected Educational Resources for Biotechnology and Microbiology

General biotechnology

'Macmillan dictionary of biotechnology' by J. Coombs (1986) Macmillan Press. ISBN 0 333 40875 6
An excellent dictionary with a very wide modern coverage and sufficient detail in the definitions to make this a useful reference to school students. Explanations are straightforward without being simplistic.

'Microbes and biotechnology' by M.R. Ingle (1986) Basil Blackwell, Studies in Advanced Biology No.4. ISBN 0 631 14455 2
Aimed specifically at 16-19 year-old students, with study guides in each chapter, specimen examination questions (and answers!) and so on. The biotechnology of the title however, is restricted to a single chapter, the rest of the book being non-applied microbiology.

'Modern biotechnology' by Sandy Primrose (1987) Blackwell Scientific Publications Ltd. ISBN 0 632 01764 3
A easily-read yet informative book with clear, uncluttered illustrations and a balanced coverage, without undue emphasis on genetic engineering.

'Biotechnology' by John Smith (1988) Revised second edtn. New Studies in Biology series, Edward Arnold. ISBN 0 7131 2960 3
This slim volume is a concise, fairly comprehensive and authoritative introduction, yet these features perhaps make this a difficult book for 16-19 year-old students to read.

'Biotechnology ... in perspective' by David Sattelle (1988) Hobsons Publishing plc. ISBN 1 85324 043 5
A colourful and stimulating introduction to biotechnology, suited to science and non-science students alike. There are questions for discussion, but readers would need to refer to other sources in order to tackle these effectively. Unfortunately no other references are given (But see *'Biotechnology in practice'* below).

'*bio*-Technology. Changing the way nature works' by John Hodgson (1989) Cassell. ISBN 0 304 31783 7
It would be wrong to describe this as a coffee table book, but the illustrations are so good, it looks like one. Fortunately the quality of the text matches that of the pictures. A one-off book for the library rather than a text or reference book.

'Micro-organisms and biotechnology' by Jane Taylor (1990) Macmillan, Science 16-19 Project. ISBN 0 333 48320 0
Not much *new* material here, but a worthy successor to previous microbiology texts aimed at school students. The information is clearly presented and gives a balanced view of the role of microbes. No other recent U.K. microbiology book aimed at this age group has so much useful material, but its biotechnology coverage is limited.

'Basic biotechnology kit' (1987)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 185 L
A practical kit with sufficient materials for six students of groups of students, including: immobilised invertase; action of pectinase on apple pulp; plant tissue culture using carrot seedlings; yoghurt, cheese and junket manufacture.

Microbiology

'Systematics and classification' by Grace Monger and Mary Sangster (1988) Longman. ISBN 0 582 35436 6
A modern general classification text (not just micro-organisms), illustrated by superb photographs.

'Monera - bacteria and cyanobacteria' (1988) - 30 minute VHS format (PAL) video recording with 40-page student booklet (photocopyable). Split into short sections for ease of classroom use. Available from Boulton-Hawker Films Ltd, Hadleigh, Ipswich, Suffolk, IP7 5BG.

'Microbial engine; algae and protozoa' (1989) - 36 minute VHS format (PAL) video recording. Available from CCAP, Institute of Freshwater Ecology, The Ferry House, Ambleside, Cumbria, LA22 0AP.
Note: A range of other resources e.g. a practical kit, is available to accompany this video.

'Bacteriology' by John Humphries (1974) John Murray. ISBN 07195 2897 6
Dated, but valuable guide to basic microbiological techniques.

'Introductory microbiology kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84400/3
Introduction to microbiological techniques, including basic culture and staining methods, and the effect of antibiotics.

'Basic microbiology kit' (1985)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 050 B
A practical package providing an introduction to basic microbiological techniques.
Slope cultures organisms are provided.

'Introductory microbiology kit' (1985)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 060 R
A practical package allowing the study of micro-organisms isolated from the environment.

'Virus transmission set' (1978)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 080 D
A practical package for demonstrating the visible effect of T4 bacteriophage on the growth of *E.coli* in liquid culture, and that viruses are small enough to pass through a fine filter, whereas bacteria will not.

'Milk testing set' (1982)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 112 J
A practical kit for conducting plate counts and resazurin tests on fresh and stale milk samples.

'Sordaria fimicola set' (1980)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 178 A
A practical package for demonstrating crossing-over in black and white-spored strains of *Sordaria*.

'Antibiotic sensitivity testing set'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84410/6
Demonstrates the sensitivity of two different species of bacteria to two different antibiotics.

'Antibiotic sensitivity set' (1982)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 136 R
A practical package demonstrating the sensitivity of *Bacillus subtilis* to antibiotics (penicillin and streptomycin).

'Introductory Trycult set'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84430/1
'Trycult' slides are pre-sterilised plastic paddles with a thin layer of selective nutrient medium on each surface. They are supplied inside screw-capped containers which both protect the medium and serve as an incubation chamber. In use the slides are dipped into a liquid such as engine oil or milk, sealed inside their transparent containers, and after a few day's incubation, microbial colonies growing on them can be observed.

'Microbial activity kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84500/7
This kit shows the various biochemical conversions carried out by three different bacteria - illustrating the breakdown of starch, protein, fat, glucose, lactose and nitrite. The media used change colour or become clear to give simple visual indications of microbial activity - including gas production.

'Microbial ecology kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84510/9
Uses selective media in sealed tubes to illustrate the role of microbial breakdown in nutrient cycling.

'Microbial breakdown kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84600/0
An introductory kit examining the breakdown of complex carbohydrates, protein, and fat by micro-organisms.

'Cellulose breakdown kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84605/9
Breakdown of carboxymethyl cellulose by both pure cellulase enzyme and extra-cellular enzymes produced by soil microbes.

'Starch breakdown kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84610/3
Breakdown of starch by *Aspergillus oryzae*.

'Pectin breakdown kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84615/2
Pectin breakdown by *Aspergillus oryzae* , pure pectinase and soil microbes.

'Fats breakdown kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84620/6
A kit examining the breakdown of fats by *Rhizopus stolonifer* and a commercial lipase.

'Protein breakdown kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84625/5
Breakdown of casein and gelatin agars by a commercial protease.

'Nitrogen cycle kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84660/7
A collection of nine practical investigations of those transformations occurring in the soil which result in the re-cycling of nitrogen and the maintenance of soil fertility.

'Nitrogen fixation kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84665/6
This kit provides the necessary media for the isolation of symbiotic nitrogen-fixing bacteria (*Rhizobium* sp.) from legume root nodules, and provides a culture of *Azotobacter* sp. (a free-living nitrogen-fixer) for examination.

'Rhizobium and clover symbiosis set'
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 146 K
A kit to study the interaction between clover (*Trifolium repens*) and *Rhizobium* sp. Involves the isolation of *Rhizobium* from clover root nodules.

'Mycorrhiza study kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84675/9
Enables the microscopic examination of ectomycorrhiza.

'Plant pathology kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84700/4
Using this kit, students can induce the plant disease brown rot (caused by *Sclerotinia fructigena*) in apples, see the symptoms in a few days, then re-isolate the fungus.

'Bacterial growth kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84520/2
Vibrio natriegens, a marine organism, grows rapidly enough for a growth curve to be determined in as little as three hours.

'Bioluminescence kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84420/9
The bioluminescent marine organism *Photobacterium phosphoreum* can be used to investigate microbial physiology. This kit enables students to observe the effect of dissolved oxygen and temperature on the bacterium's growth.

'Fermentation' Acorn BBC microcomputer program. Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number A25825/8
Simulation allowing the effects of temperature, substrate concentration etc. to be varied and their effects on microbial growth to be seen either in batch or continuous culture. Data plotted as graphs on screen.

'Yeast growth' Acorn BBC microcomputer program. Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number A25830/1
Simulates cell count investigation using haemocytometer grid.

Tissue culture

'Harris animal tissue culture kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M83580/4
Uses kidney cells of *Xenopus* sp. Growth (mitosis) and contact inhibition may be observed.

'Meristem tissue culture kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M83905/6
Provides all the necessary media for the propagation of plantlets from cauliflower curd.

'Carrot tissue culture kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M83910/9
Demonstrates the production of callus tissue from a root explant, and the recovery of whole, cloned carrot plants from the callus.

'Tobacco tissue culture kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M83915/9
Taking tobacco leaf material as the source of the explant, this kit allows students to grow tobacco callus and subsequently experiment with changing the levels of plant growth substances in the medium in order to produce roots or shoots.

'Plant tissue culture Set 1' (1983)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 190 L
An introductory set demonstrating the techniques and potential of plant tissue culture. The students: culture carrot seedlings asptically; grow callus tissue; initiate a suspension culture; develop many new plantlets from the callus.

'Plant tissue culture Set 2' (1983)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 200 U
A kit to show the effects of different concentrations of plant growth substances on root, shoot and callus growth.

'Leaf protoplast kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M83953/6
Protoplasts made by enzymic removal of cell walls from broad bean leaf tissue.

'Basic techniques of plant tissue' 26 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20950/4
An introduction to the initiation, establishment and maintenance of callus and cell suspension cultures using a variety of plant species.

Genetic engineering

'In search of the double helix' by John Gribbin (1985) Corgi Books. ISBN 0 552 12656 X
An easy-to-read yet highly informative and enjoyable book, giving an essential historical perspective to the developments of molecular biology.

'The cartoon guide to genetics'by Larry Gonick and Mark Wheelis (1983) Harper and Row (Barnes and Noble Books). ISBN 0 06 460416 0
Fun-to-read but highly informative comic book suitable for even the most disillusioned students.

'Reshaping life' by G.J.V. Nossal and Ross L. Coppel (1989) Revised second edtn. Cambridge University Press. ISBN 0 521 38969 0
A lucid and authoritative account of genetic engineering and associated developments, suitable for the informed layman. Tackles some of the social and moral issues. Clearly illustrated.

'Genetic engineering' by Paul Wymer (1988) Hobsons Publishing plc. ISBN 1 85324 160 1 (Minimum order 10 copies)
A brief treatment of nucleic acid structure and protein synthesis leading to some of the essential techniques of genetic engineering.

'Genetic mechanisms' by M.R. Ingle (1986) Basil Blackwell, Studies in Advanced Biology No.2. ISBN 0 631 90950 8
Reasonably-priced, but somewhat turgid text aimed at 16-19 year-old students.

'Applied genetics' by Geoff Hayward (1990) Macmillan, Science 16-19 Project. ISBN 0 333 46659 4
An excellent modern text for school students.

'Genetic engineering' Acorn BBC/RML Nimbus/IBM/Acorn Archimedes computer program. Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ.
Simulation of main procedures involved in genetic engineering. Reviewed in Journal of Biological Education, 23 (3) pp.218-222.

'Genetic engineering' 15 minute VHS format (PAL) video recording made for employees of Novo Nordisk Bioindustries Ltd., but suitable for older school students. Available from Philip Harris Ltd, Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number P00104/7

'Genetic transfer kit' (1985)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 236 J
Uses *Agrobacterium rhizogenes* to introduce new genetic material (a tumour-inducing plasmid) into carrot slices.

'Plant tumour kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84705/3
Agrobacterium tumefaciens is used in this kit to induce crown gall disease in sunflower seedlings.

'Introduction to natural genetic engineering kit' (1984)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 240 P
Transfer of antibiotic resistance plasmid from one strain of *E.coli* to another. Uses riplamycin and chloramphenicol.

'DNA extraction kit' (1987)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 245 Y
Extraction of DNA from *E.coli*.

'Genetic engineering' 12 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M39835/0
Basic techniques of bacterial genetic engineering.

Industrial uses of enzymes

'Enzymes and their role in biotechnology' by Paul Wymer and A.R. Teal (1988) The Biochemical Society, 59 Portland Place, London W1N 3AJ. Tel: 071 580 5530
One of a series of five introductory booklets aimed at school students produced by the *Biochemical Society*.

'Enzymes in industry and medicine' by Gordon Bickerstaff (1987) New Studies in Biology series, Edward Arnold. ISBN 0 7131 2935 2
A comprehensive guide to the many industrial uses of enzymes.

'Enzymes at work' Novo Nordisk Bioindustries Ltd. Available free of charge.
Commercial enzymes brochure, now slightly out-of-date.

'Enzyme production set' (1983)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 220 G
A set to show the production of three commercially important enzymes. They are protease (*Bacillus subtilis*); invertase (*Saccharomyces cerevisiae*) and amylase (*Aspergillus oryzae*).

'Enzymes at work kit' (1985)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 215 G
Sufficient materials for demonstrating the following applications of enzymes to six students or groups of students: amylase in malting and brewing; invertase in soft centred confectionery; pectinase for fruit juice extraction; cellulase in cellulose degradation; proteases in biological detergents.

'Enzyme and cell immobilisation set' (1985)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 222 R
This set allows students to explore the production and use of immobilised enzymes and cells. The chemical and physical properties of yeast cells immobilised in calcium alginate can be investigated, and the activity of free and immobilised invertase can be compared.

'Immobilised enzyme kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M85910/9
Examines entrapment of yeast cells in calcium alginate and their use in fermentation, immobilised glucose isomerase, and the preparation and use of immobilised invertase.

'Build a bioreactor kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M85915/8
Enables a simple immobilised enzyme bioreactor using ß-galactosidase to be constructed.

'Enzymes in the home' (1984)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 224 F
A kit to show the domestic uses of enzymes. The use of 'rennet' in dairy products and proteases in biological washing powders and meat tenderisers is investigated.

'Enzymes in industry' (1984)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 226 Q
This kit illustrates some of the many industrial uses of enzymes. It examines the use of pectinases in fruit juice processing, the use of 'rennet' in the cheese industry, and recovery of silver from photographic film using proteases.

'Enzyme biotechnology kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M85900/6
Experiments included in this kit are: rennet in cheese production; proteases in meat tenderisers and biological washing powders and pectinase in fruit juice production.

'Enzymes in starch hydrolysis and sweetener production' (1981)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 228 E
Enables the production of high fructose corn syrup from maize starch using amylase, amyloglucosidase and immobilised glucose isomerase.

Industrial fermentation

'Finding out... about brewing technology' by Bill Down (1988) Hobsons Publishing plc. ISBN 1 85324 180 6 Written for younger students, but suitable background information for 16-19 year-olds.

'Naturally it's beer' (1988) - 30 minute VHS format (PAL) video recording, produced by the Brewer's Society and available to schools through Guild Sound and Vision Ltd, 6 Royce Road, Peterborough, PE1 5YB.
Explains how beer is made.

'Citric acid. A study of industrial fermentation' (1988) - 17 minute VHS format (PAL) video recording. Available from North Yorkshire Schools and Industry Association, Park Grove School, Dudley Street, York, YO3 7LG.
A 70-page booklet of data interpretation and practical work is also available from the above address.

'Applications of microbes in the dairy industry' (1990) Hobsons Publishing plc. ISBN 1 85324 043 5 (minimum order 10 copies).
Produced in association with Express Foods Ltd. (A major dairy food manufacturer). Mostly about yoghurt and Stilton cheese.

'Lactic acid fermentation set' (1983)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 230 W
A set to show the changes which occur in milk during the production of yogurt. students can investigate the production of and effects of acid, the growth of bacteria and changes in dissolved oxygen (using resazurin test).

'Microbiology of yoghurt kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84530/5
This kit investigates the differential growth rates of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in yoghurt production; allows the total bacterial population of the yoghurt to be estimated; enables the clotting (isoelectric) point of casein to be determined, and the pH of the developing yoghurt to be monitored.

'Cheesemaking' 25 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20830/5
The processes involved in the small-scale production of cheese are described in detail.

'Food from microbes, Part 1' 57 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20826/3
The production of many familiar food using microbes.

'Food from microbes, Part 2' 47 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20827/5
The potential use of microbes to convert natural resources into valuable foods.

'Penicillin' 33 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20823/8
A detailed account of the discovery, commercial development and theraputic effect of penicillin.

'Methane production' 25 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20826/3
Bacterial methane production from waste.

'Fermentation kit' (1984)
Available from Griffin and George, Bishop Meadow Road, Loughborough, Leicestershire, LE11 0RG. Catalogue number ZEA 210 N
The kit contains the basic materials needed to show the processes used to produce beer.

'Alcohol fermentation kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84535/4
Both aerobic and anaerobic fermentation are covered by this kit, which also includes a comparison of the activity of free and immobilised yeast cells.

'Bacterial products kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84540/8
This kit demonstrates the production of dextran (a polysaccharide used in the pharmaceutical industry) by *Leuconostoc mesenteroides*.

'Bacterial leaching kit'
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M84550/0
Demonstrates bacterial leaching of coal samples containing pyritic sulphur using *Thiobacillus ferroxidans*. Also illustrates the principles of bacterial leaching which are used in mining e.g. of copper from low-grade ores.

'Mineral biotechnology' 25 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9JJ. Catalogue number M20900/0
Includes bacterial leaching of mineral sulphides and coal and the treatment of effluents to recover useful materials.

'Treatment of sewage and other used waters' 44 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20829/9
Biological treatment processes in a typical sewage works.

Safety

'Microbiology. An HMI guide for schools and non-advanced further education' (1985) HMSO. ISBN 0 11 270578 2
Essential reading for United Kingdom teachers. Does not adequately cover use of fermenters in school, nor aspects of biotechnology other than traditional school microbiology.

'Topics in safety' (1988) Revised second edtn. ISBN 0 86357 104 2 Available from: Association for Science Education, College Lane, Hatfield, Herts. AL10 9AA.
Includes a useful safety risk assessment flow chart and updated details of hazards associated with fermenters, enzymes and plant growth substances.

'Safe handling of micro-organisms' 43 frame 35mm slide set
Available from Philip Harris Biological Ltd., Oldmixon, Weston-super-Mare, Avon, England, BS24 9BJ. Catalogue number M20750/7
Sponsored by the Microbiology in Schools Advisory Committee (MISAC). Somewhat dated, but the basic procedures are still valid.

Texts describing practical work

'Plant tissue culture' by Tony Storr (1985) Association for Science Education, College Lane, Hatfield, Herts. AL10 9AA. Experimenting with Industry series No.13. ISBN 0 86357 031 3
Essential reading for anyone wishing to try plant tissue culture in school. There's no point in buying a kit if you have this book, since it gives complete details of the materials required and the manufacturers who supply them in the United Kingdom.

'Practical microbiology and biotechnology for schools' by Paul Wymer (1986) Macdonald Educational. ISBN 0 356 11566 6
30 photocopyable workcards and a teacher's guide. Aimed at 14-16 year-olds, but with plenty of very well tested practical exercises which could easily form the start of more demanding investigations by older students. Produced in association with the Society for General Microbiology, so the emphasis throughout is on good practice.
(Now available direct from the National Centre for Biotechnology Education)

'Biotechnology - a resource book for teachers' by John Dunkerton (1989) Association for Science Education, College Lane, Hatfield, Herts. AL10 9AA. . ISBN 0 86357 111 5
Brief suggestions for original practical work and a useful resource list for teachers.

'Biology and Industry. Practical biotechnology for A-Level' by Irena Olejnik and Bev Farmer (1989) Blackie. ISBN 0 216 926 27 0
Photocopyable material and suggestions for practical work backed up by additional information. Be warned that some of the practical work requires specialist facilities/organisms.

'Biotechnology in practice' by Paul Wymer (Ed.) (1990) Hobsons Publishing plc. ISBN 1 85324 315 9

21 photocopyable investigations covering a wide range from simple culturing to plasmid extraction. Extensive background information and resource lists are included.

'Biotechnology Module' by Peter Screen (Co-ordinator) (1989) Warwick Process Science Project. Ashford Press Publishing, 1 Church Road, Shedfield, Hants. SO3 2HW. ISBN 1 85253165 7

Intended for younger students, but with numerous suggestions which are likely to prove useful starters for more advanced investigations.

NCBE *Newsletter* published three times a year features numerous suggestions for practical work. Available from the National Centre for Biotechnology Education, Department of Microbiology, University of Reading, London Road, Reading, RG1 5AQ, United Kingdom.

This list of selected educational resources for biotechnology and microbiology suitable for students aged 16 to 19 was prepared by the

NATIONAL CENTRE FOR BIOTECHNOLOGY EDUCATION
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Appendix 2

A Collection of Information on Safety Issues in School-based Biotechnology

A. Safety in School Science: Biotechnology (Source: *Education in Science*, Association for Science Education, U.K., January 1988)

Biotechnology adds a new and fascinating dimension to courses in biology and technology, and many simple, safe investigations can be carried out in the school laboratory. In most cases, the hazards are the usual ones encountered in practical work in biology or chemistry, such as the handling of microorganisms and chemicals. Biotechnology does present a few less familiar problems, which are outlined here.

While many of the practical activities in which risks arise in biotechnology may be "new," the nature of the risks is well known. However, in view of the possible scale of such practical activities, the effects of an accident may be more widespread and of considerable magnitude. Types of activity and the source and nature of these possible risks are tabulated in table 1.

Work with microorganisms in schools and colleges has been defined on three levels in *Topics in Safety*[1], and more recently by HMI in *Microbiology. An HMI guide for schools and non-advanced further education*[2]. Note that these levels are *not* the same definitions as the Containment Levels used internationally by professional microbiologists. Level 1 includes work with organisms which have little, if any, known risk such as some yeasts, molds, algae, and protozoa. This work can be supervised by teachers with no specialist training unlike that at Levels 2 and 3, for which this training is considered essential. Level 2 work includes the use of appropriate culture media and incubation conditions using known organisms, including selected bacteria and those cultured from the environment. Level 3 work includes, in addition to that at Level 2, subculturing and transfer procedures requiring good aseptic technique.

Fermentation

Fermentation may be carried out on a small or relatively large scale in a fermenter, either constructed within the school, or commercially produced. After initial inoculation, large numbers of microorganisms may be produced. Using a fermenter may require electrical connection, and gases may be produced, some of which may be flammable.

Source of Risk	Nature of Risk	Type of Activity in which Risks may arise	Limitations of Risk in Schools
Microorganisms: -bacteria -fungi	infection allergy	fermentation technologies enzyme technologies illustrations of principles of genetic engineering	choice of organism (allergenic and infective organisms not used) obtain only from specialist schools' suppliers
Gases	explosion pressure build up flammability toxicity	fermentation technologies	venting of fermenters relatively small quantities choice of organism
Electricity	electrical shock source of ignition fire hazard	fermentation technologies other practical work using mains electricity	low voltages–regular maintenance careful design
Enzymes	allergy	enzyme technology	laboratory technique
Antibiotics	sensitization allergy production of resistant organisms	background basic microbiology production in fermentation technologies	restrict use of antibiotics avoid use of antibiotic-producing organisms careful disposal of cultures exposed to antibiotics
Plant cell culture	toxicity (of growth substances)	"tissue" culture	laboratory technique
Plant growth substances	toxicity	investigations on plant growth plant tissue culture biotransformation	laboratory technique as for other toxic chemicals
Radiation (UV)	tissue damage	investigations illustrative of the principles of genetic engineering	avoid short-wave UV
Animal cell cultures	risks of serious infection -bacterial -fungal -viral mycoplasma contamination	"tissue " culture	risk limitation difficult, so not recommended. If carried out, use only cultures obtainable from specialist schools' suppliers in UK aseptic technique
Human body fluids	risk of infection	enzyme demonstrations immunology	do not use human blood or cell samples

Risks from fermentation can be minimized by choosing suitable organisms and techniques, using safe ways of handling suitable microorganisms, and keeping the volume of the medium to a practical minimum.

Microorganisms particularly suitable for studying fermentation technology at school level are yogurt-making bacteria, strains of non-pathogenic yeast, and some unicellular algae, for example: wine yeast, beer yeast, breadmaking yeast, dried yogurt cultures.

For work at a more advanced level, (i.e., Level 2 and Level 3), it is recommended that only microorganisms with unusual growth requirements are used, such as those requiring high salt, acid conditions, low or high temperatures (i.e., as far away from 37°C as possible). This tends to encourage growth of the "intended" organism at the expense of undesirable contaminants.

Examples of bacteria which have unusual growth requirements (and which are included in the HMI list[3]) include: *Vibrio nutriegens (Beneckea nutriegens), Photobacterium phosphoreum, Acetobacter aceti*.

Organisms other than those listed above may be used, but only if the teacher responsible has had appropriate training in microbiological techniques, i.e., giving a good knowledge of aseptic techniques, subculturing, recognition of contamination, and safe disposal.

Cultures should be obtained only from recognized specialist UK schools suppliers. They should never be exchanged between schools, colleges, and other institutions. Maintenance beyond two or three subcultures should be carried out only if those involved have had training in the necessary techniques.

The use of suitable techniques is particularly critical when working with fermentation:

-prepared and sterilized media and equipment must be used;
-aseptic technique must be used when inoculating the fermenter, and when taking samples;
-the volume of actively growing inoculum should be a significant fraction (e.g., 20%) of the volume of the medium. Thus, any chance contaminant getting in during the inoculation procedure has to compete with an established organism;
-spillages should be avoided, and if they happen, need to be carefully managed; and
-materials and equipment must be safely sterilized and disposed of after use.

Any spillages must be cleared up immediately using a suitable disinfectant (such as clear phenolic, *Clearsol, Hycolin, Puritol, Stericol,* or *Sudol* being readily available examples.) All disinfectants should be freshly prepared, and used at manufacturers'

recommended dilutions. Spills on clothing may be best disinfected using a surface-active disinfectant (Harris "BAS cleaner" and Griffin ASAB are readily available examples). Any spillage may cause the formation of an aerosol, which may remain for some time. If a gross spillage occurs, HMI recommend[2] that the laboratory should be cleared immediately and that all accidental microbiological spillage incidents should be recorded.

Aerosols are fine sprays or suspensions which in this context contain microorganisms. The scale of liquid culture involved may increase the chance of any contaminant being cultured in large numbers and then being released back into the environment by the formation and escape of aerosols. A fermenter must be adequately vented to prevent the build up of pressure in the vessel. For work with yeast and similar organisms, a wine-making trap or substantial non-absorbent cotton wool plug attached to the air vent should be sufficient to trap any fine spray. For more demanding work, the use of in-line microbiological filter cartridges should be considered. In solid-culture fermentation, e.g., cheese, tempe, there is the possibility of fungal spores being produced in quantities sufficient to produce sensitization and possible allergic reactions.

Fermenters may need to be heated and stirred, and much useful information can be gained by the use of monitoring equipment ranging from stand-alone instruments to computer data-logging packages. A fermenter assembly with a forest of wires can therefore easily appear daunting electrically. However, most of these will be at less than 20 volts, which is relatively safe. Care should be taken to keep mains and other leads tidy, and it is clearly wise to site electrical equipment as far as is possible from the fermenter vessel and wet working areas. Mains-powered equipment (such as a pH meter) used with a fermenter should be of a reputable commercial design intended for school use. Where apparatus other than purely proprietary devices intended for continuous operation (e.g., incubators) is left running out of school time, then it should bear a warning notice.

When using fermenters, it is also important to remember that relatively large volumes of potentially hazardous substances, e.g., antibiotics, enzymes, pesticides, or growth substances, can be produced, posing problems for dealing with spillages or safe disposal.

Some schools are interested in investigating "biogas" generation by the fermentation of silage, grass clippings, etc. Methane is produced slowly when the mixture is inoculated by the addition of well-rotted garden compost or rich pond mud (and the presence of broad bean husks is helpful). Such procedures are acceptable, provided the methane is handled with the care due to a flammable gas. However, we do *not* recommend the use of animal manure as an inoculum, although of course it is widely used both in the Third World and in the UK for fuel gas production. While many people handle horse manure in stables or when gardening, deliberate culturing in a fermenter may introduce unacceptable risks of infection (for example, from *Salmonella sp*), and thus the use of animal manure is best regarded as Level 2 work in this context.

Disposal

All cultures and their containers should be sterilized using an autoclave before disposal. The culture medium can be then poured away down a sink, which is flushed with a large volume of water.

Antibiotics

Antibiotics can cause allergic reactions, and it is now recommended that penicillin-producing cultures should not be used in schools. Culturing of large quantities of any microorganism which produces an antibiotic could be hazardous. There is growing concern over the promiscuous use of antibiotics and the increasing prevalence of antibiotic-resistant strains of microorganisms.

Plant-Growth Substances

Plant-growth substances (known wrongly as plant hormones) are extensively used both in growth investigations and in plant tissue culture. Many are toxic, a few may also be carcinogenic. They are normally used in very low concentrations in experiments and media, but technicians and teachers handling solids or more concentrated solutions should be aware of the hazards, and take appropriate precautions, e.g., by wearing disposable gloves.

Enzymes

Hazards from enzymes are associated with their increased use both in quantity and variety. Teachers and technicians should be aware of the hazards involved in handling enzymes in solid and liquid form, to avoid spillage and the formation of aerosols. It must always be remembered that enzymes are biologically active proteins which can irritate the skin or eyes and cause allergic reactions.

Animal Tissue Culture

Practical work with animal tissue cultures is not recommended for use in schools because of the risk of serious infection. If such work is to be carried out, ensure that the culture is obtained from a recognized specialist UK schools supplier.

Genetic Engineering

Finally, it is important to consider what aspects of genetic engineering can be explored in schools. Guidance issued by HMI[2] effectively proscribes practice of the "techniques of genetic engineering" in schools.

This general proscription is doubtless based on knowledge of requirements or regulations made under the Health and Safety at Work Act. However, "genetic engineering" is a loose and somewhat general term. The Health & Safety Executive (HSE) regulations and guidance adopt a more precise definition of the term "genetic manipulation."

"Genetic manipulation" is defined by HSE as:

> (the) formation of new combinations of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into any virus, bacterial plasmid, or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation.

The HSE regulations and guidelines[4] require registration and notification for such activities as well as the setting up of a local safety advisory committee. Schools and non-advanced FE institutions are not debarred from such arrangements. However, the HSE requirements would prove onerous for schools. Taken together with the DES guidance, they would suggest that schools would be ill-advised to attempt practical work involving such techniques.

As well as taking account of what is "allowable" under the HSE arrangements, a prudent safety policy must consider also what is educationally desirable. There are many practical activities, some already done in schools, which allow illustration of the basic genetic, biochemical and microbiological principles which underpin "genetic engineering." So long as these remain analogous to rather than homologous with parts only of the techniques of "genetic manipulation" they may be acceptable.

For example, there are investigations which allow illustration of plasmid transfer. However no *new* nucleic acid molecules are involved and transmission of the new characteristics to another generation does not occur. Other illustrative investigations involve plant tumour induction and transfer of natural antibiotic resistance between bacterial strains. The HMI guidance proscribes deliberate induction of mutation in bacteria; however, the induction of mutation in yeasts using UV radiation is still considered acceptable.

Sources of General Information

The Association for Science Education, College Lane, Hatfield, Herts. AL109AA.
CLEAPSE School Science Service, Brunel University, Uxbridge, UB83PH.
MISAC (Microbiology in Schools Advisory Committee), the Institute of Biology, 20 Queensberry Place, London SW72DZ.

National Centre for School Biotechnology Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ.
The Scottish Schools Science Equipment Research Centre, 103 Broughton Street, Edinburgh EH1 3RZ.

References and Useful Publications

1. *Topics in Safety* (Association for Science Education, 1982). A collection of safety articles reprinted from *Education in Science*.

2. *DES, Microbiology. An HMI guide for schools and non-advanced further education* (HMSO, 1985).

3. Education Service Advisory Committee; *Educational Establishment--unattended operation of experimental apparatus outside normal working hours* (Health & Safety Commission, 1986).

4. *Genetic Manipulation Health & Safety Commission Regulations and Guidance* (HMSO, 1978).

5. Interdepartmental Committee on Biotechnology, *Biotechnology. Support and Regulations in the UK. A plain man's guide*. (The Laboratory of the Government Chemist/Department of Trade and Industry, 1986).

6. C. H. Collins (ed.), *Safety in Biological Laboratories*, (Institute of Biology/John Wiley & Sons, 1985).

7. NCSB Newsletter. Published by National Centre for School Biotechnology, Department of Microbiology, University of Reading, Berks. RG1 5AQ.

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B. Biosafety (Source: *Advances in Genetic Technology*. BSCS, Lexington, Massachusetts, USA: D.C. Heath, 1988).

A biohazard is a replicating organism or biological substance--arising from the laboratory or work environment--that may be hazardous to the health of the individual laboratory worker or to the community. Such biological organisms and substances include infections or parasitic agents, as well as noninfectious organisms. The noninfectious organisms include some fungi, yeasts, algae, plants, plant products, animals, and animal products that might cause occupational diseases.

These organisms are grouped into classes according to their perceived or potential hazard to humans. The risk associated with the use of the organisms in each class is controlled by two containment approaches: (1) physical containment involving safe laboratory practices, laboratory design, and equipment; and (2) biological containment, which involves the use of organisms that pose little or no risk to the environment.

Certain precautionary procedures are used in handling microorganisms, cells in culture, organisms containing recombinant DNA molecules, and suspected or known hazardous organisms. The only federal or state regulations governing the handling of potential biohazards are the U.S. Department of Agriculture (USDA) regulations on animal care, the U.S. Department of Transportation (DOT) regulations on shipping, and the U.S. Environmental Protection Agency (EPA) interim guidelines for applications involving field testing of engineered microbial pesticides. Research laboratories voluntarily comply with the laboratory safety aspects of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules, and the NIH Guide for the Use of Laboratory Animals. The procedures for handling laboratory materials in this program incorporate safety concepts and guidelines published by NIH, the Centers for Disease Control (CDC), the USDA, the American Industrial Hygiene Association (AIHA), and the Medical Research Council of Canada (MRCC).

There are four levels of physical containment--Biosafety Levels BL1, BL2, BL3, and BL4 (designations of the NIH), BL4 includes organisms of greatest potential risk to humans. The organisms used in this program, *Agrobacterium tumefaciens* and *Escherichia coli* JM103(or K-12) containing a pBR322 plasmid, are classified BL1, because those organisms pose little threat to human health. However, physical containment to prevent environmental release is necessary. **You must follow these containment procedures.**

1. The laboratory door must be kept closed, except as needed for access. No special laboratory design or special containment equipment is required to work with BL1 organisms.

2. There is to be no eating, drinking, smoking, or storage of food in the laboratory.

3. Workers must wash their hands thoroughly after handling organisms, and upon leaving the laboratory.

4. Pipetting by mouth is prohibited; use mechanical pipetting devices only.

5. Take special care to avoid skin contamination with potentially biohazardous organisms. Wear gloves when skin contact with the agent is unavoidable.

6. Avoid use of hypodermic needles and syringes when alternative methods are available. Take care to minimize the creation of aerosols or splatters that occur when a hot loop or needle is inserted into a culture, an inoculating loop is flamed so that it splatters, or fluids are forcefully ejected from pipettes and syringes.

7. Separate biological waste from office trash and general laboratory trash.

8. Collect solid and liquid biological waste in specially marked containers (such as orange autoclave plastic bags or biosafety disposal bags) and disinfect by autoclaving or by treatment with a solution of commercial bleach. The containers should be airtight and must be secured tightly and marked with tape that reads "AUTOCLAVED" after autoclaving.

9. Solutions useful for decontamination include chlorine-based disinfectants, which are effective against vegetive bacteria, most viruses, and fungi at 500 ppm. *Clorox* or *Purex* diluted at 1:100 provide the necessary concentrations. A dilution of 1:20 yields 2500 ppm, which will also kill bacterial spores. Ethanol (70-80 percent) in water is effective against vegetative bacteria and nonlipid-containing viruses and is effective for surface decontamination.

10. Solid trash that has been contaminated by biological waste must be collected in a separate, specially-marked disposal bag (see step 8). Package all sharp instruments (needles or scalpel blades inside the container) in separate cardboard containers or in other commercially available containers for disposal. No pipettes should protrude from the disposal bag. Free liquids and gels must be absorbed by paper towels to minimize the risk of leakage. The bags must be secured tightly, and tape that shows the word "AUTOCLAVED" after autoclaving must be clearly visible on the bags.

11. Contaminated materials that are to be decontaminated at a site away from the laboratory must be placed in a durable, leak-proof container that is closed prior to removal from the laboratory. Contaminated materials that are to be reused should be decontaminated prior to washing.

12. Laboratory workers, students, and teachers must clean and decontaminate (with 70 percent ethanol) work surfaces. Those individuals also are responsible for the decontamination of biological material spills, removing contaminated supplies (such as pipettes and syringes) from floors, and decontamination of contaminated floor surfaces.

C. Ten Rules for Safe Microbiology and Biotechnology in School (Source: Eckhard R. Lucius, IPN, Kiel, West Germany)

In the Federal Republic of Germany, the safety of scientific teaching is governed by guidelines set out by the Ministers of Education'. The code of conduct which generally applies to scientific experiments is expounded here, e.g., no eating, drinking, or smoking in laboratories, chemicals should not be tasted, hands should be washed, pipetting should not be carried out using mouth. The IPN recommends that the following ten rules should also be observed for microbiological and biotechnical experiments:

1) Pure cultures of microorganisms suitable for use in schools should be ordered from the DSM (international: from the ATCC - American Type Culture Collection).

2) Enrichment plates of microorganisms from the environment should be sealed by sticking the slit between the base and lid of the petri dish together with adhesive tape.

3) Bacteriological experiments with fecal material ("biogas from sewage sludge") should not be carried out.

4) Work in sterile conditions; i.e., nutrient solutions should not only be boiled, but sterilized in a pressure cooker or autoclave. Glass equipment or pipettes which come into contact with sterile solutions should be sterilized for 30 minutes in a drying cupboard at 180°C.

5) Colonies of mold which have formed spores should not be examined under the microscope without using petri slides.

6) Do not use "antibiotics from the chemist's" so that human germs cannot become resistant to antibiotics on prescription.

7) Use disposable petri dishes instead of glass petri dishes.

8) Mouth pipetting should never be employed. A piston device should be used to draw the liquid up the pipette instead of a rubber bulb.

9) Do not leave bacterial cultures which are no longer required lying around. Old cultures should be destroyed in a pressure cooker and disposed of.

10) Only use microbial nucleic acids if they occur naturally in the organism (in-vivo-DNA). Do not use genetically engineered material (in-vitro-DNA).

D. List of Microorganisms Suitable/Unsuitable for Use in School (Bacteria and Fungi)
(Source: Eckhard R. Lucius, IPN, Kiel, West Germany)

Suitable Bacterial and Fungal Strains:

Species	**DSM-Nr.**	**Possible Experiments, Special Properties:**
Acetobacter aceti (B)	3508	Producing acetic acid
Agrobacterium tumefaciens (B)	30205*	Inducing tumors in plants
Bacillus licheniformis (B)	13*	Decomposing protein
Bacillus mycoides (B)	2048*	Specific tendency to form colonies
Bacillus megaterium (B)	32*	Specific cell size
Bacillus subtilis (B)	10*	Decomposing starch
Candida utilis (F)	2361	Producing biomass
Cellulomonas uda (B)	20107*	Decomposing cellulose
Erwinia carotovora (B)	30168*	Decomposing phospholipids
Escherichia coli K 12 (B)	498	Genetic experiments on bacteria
Lactobacillus bulgaricus (B)	20081*	Producing yogurt, curds
Lactococcus lactis (B)	20481*	Producing yogurt, curds
Leuconostoc mesenteroides (B)	20343*	Producing sauerkraut
Micrococcus luteus (B)	20030*	Specific colony color
Penicillium camemberti (F)	1995	Producing cheese, microskopy
Penicillium roqueforti (F)	1079	Producing cheese, microskopy
Phycomyces blakesleau. (F)	1359	Heterogenesis
Pseudomonas fluorescens (B)	50090*	Decomposing gelatine
Rhizobium leguminosarum (B)	30132*	Nitrogen fixation
Rhodotorula rubra (F)	70403	Colored Yeast Colony
Saccharom. cerevisiae (F)	70449*	Experiments with Yeast
Sporosarcina urea (B)	2281*	Decomposition of urea
Streptomyces griseus (B)	40236*	Producing antibiotics
Trichoderma viride (F)	63065	Decomposing Cellulose
Vibrio natriegens (B)	759*	Microbia growth
Xanthomonas campestris (B)	1706	Producing biopolymer

Unsuitable Bacterial and Fungal Strains:

Aspergillus nidulans (F)
Aspergillus niger (F)
Aspergillus oryzae (F)
Bacillus cereus (B)
Chromobacterium violaceum (B)
Clostridium perfringens (B)
Mucor ssp. (F)
Penicillium notatum (F)
Photobacterium phosporeum (B)
Proteus vulgaris (B)
Pseudomonas aeruginosa (B)
Pseudomonas solanacearum (B)
Pseudomonas tabacci (B)
Serratia marcescens (B)
Staphyloccus aureus (B)
Vibrio fischeri (B)
Xanthomonas phaseoli (B)

Note: (B) Bacterial Species, (F) Fungi
Strains not listed must be enquired about separately.

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